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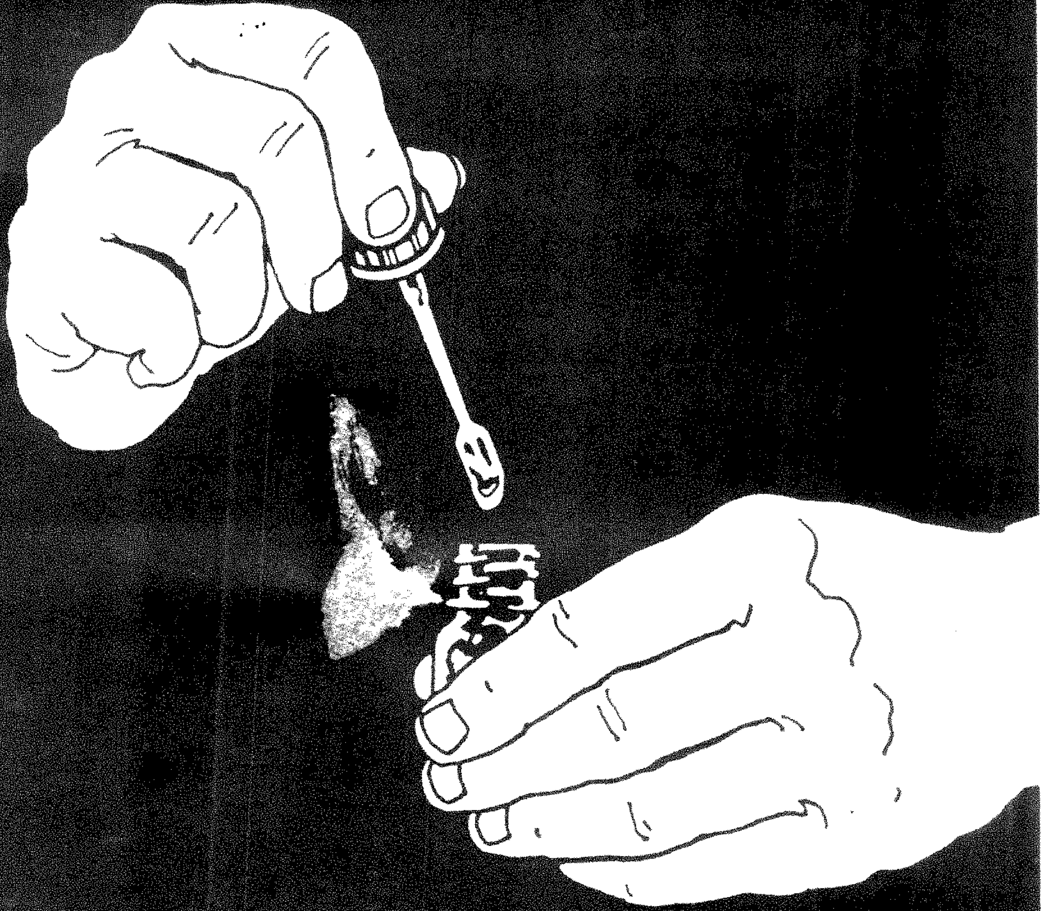

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Refs: Practitioner, 207, 197, 1971.
Practitioner, 209, 453, 1972.

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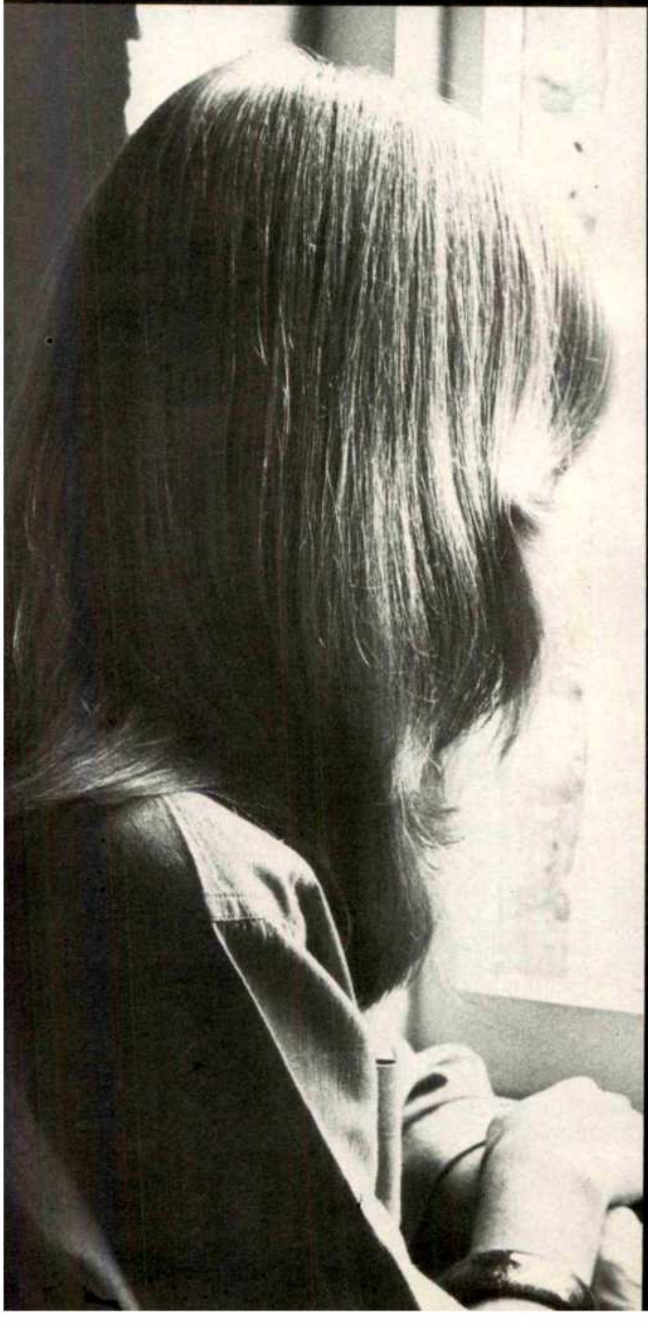

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Brit J Derm, (1971), **84**, 366.

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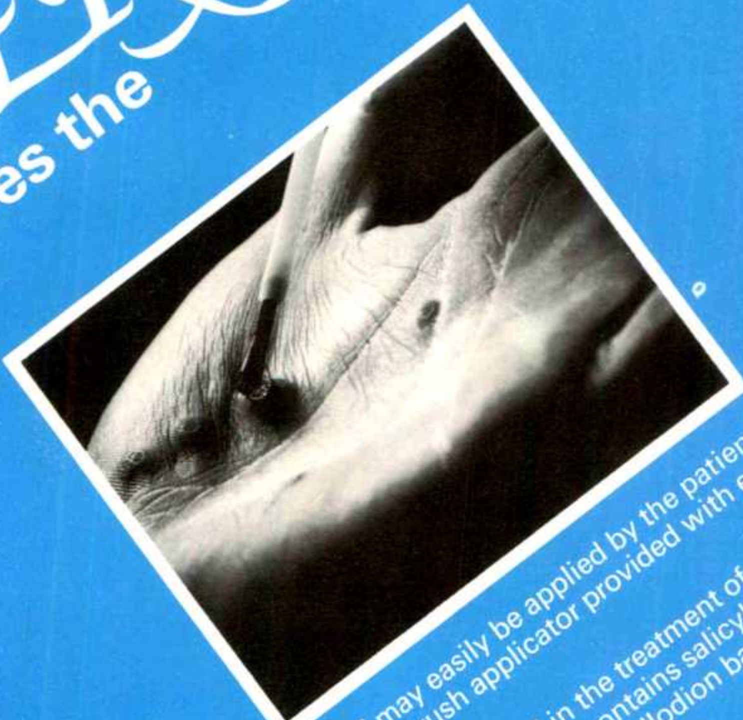

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*British Medical Journal (1975) 3, 626

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HISTOPATHOLOGY OF THE SKIN

Walter F. Lever M.D. and Gundula Schaumburg-Lever M.D. *Fifth Edition*, 1975. 816 pages, 436 illustrations (8 colour). Lippincott, £36.40

In the eight years that have passed since publication of the fourth edition so much new information has become available on nearly every major dermatosis that the book had to be entirely rewritten. The greatest changes were required in the description of the lymphomas because they have been reclassified in recent years. Many changes have been made also in the description of the bullous dermatoses and of the metabolic diseases, especially of amyloidosis, colloid milium, hyalinosis cutis et mucosae, and porphyria. Also the division of malignant melanoma in situ into two types with different prognosis has been given due consideration.

More than 20 diseases not mentioned in the fourth edition are described in this edition. Some of them are newly recognized entities, such as disseminated superficial actinic porokeratosis, perforating folliculitis, reactive perforating collagenosis, angiolymphoid hyperplasia with eosinophilia, infantile digital fibromatosis, trichilemmoma, and malignant eccrine poroma. Also several previously known diseases because of their increased interest for the dermatologist have been incorporated, among them regional enteritis (Crohn's disease), Tangier disease, lipogranulomatosis (Farber's disease), and generalized eruptive histiocytoma.

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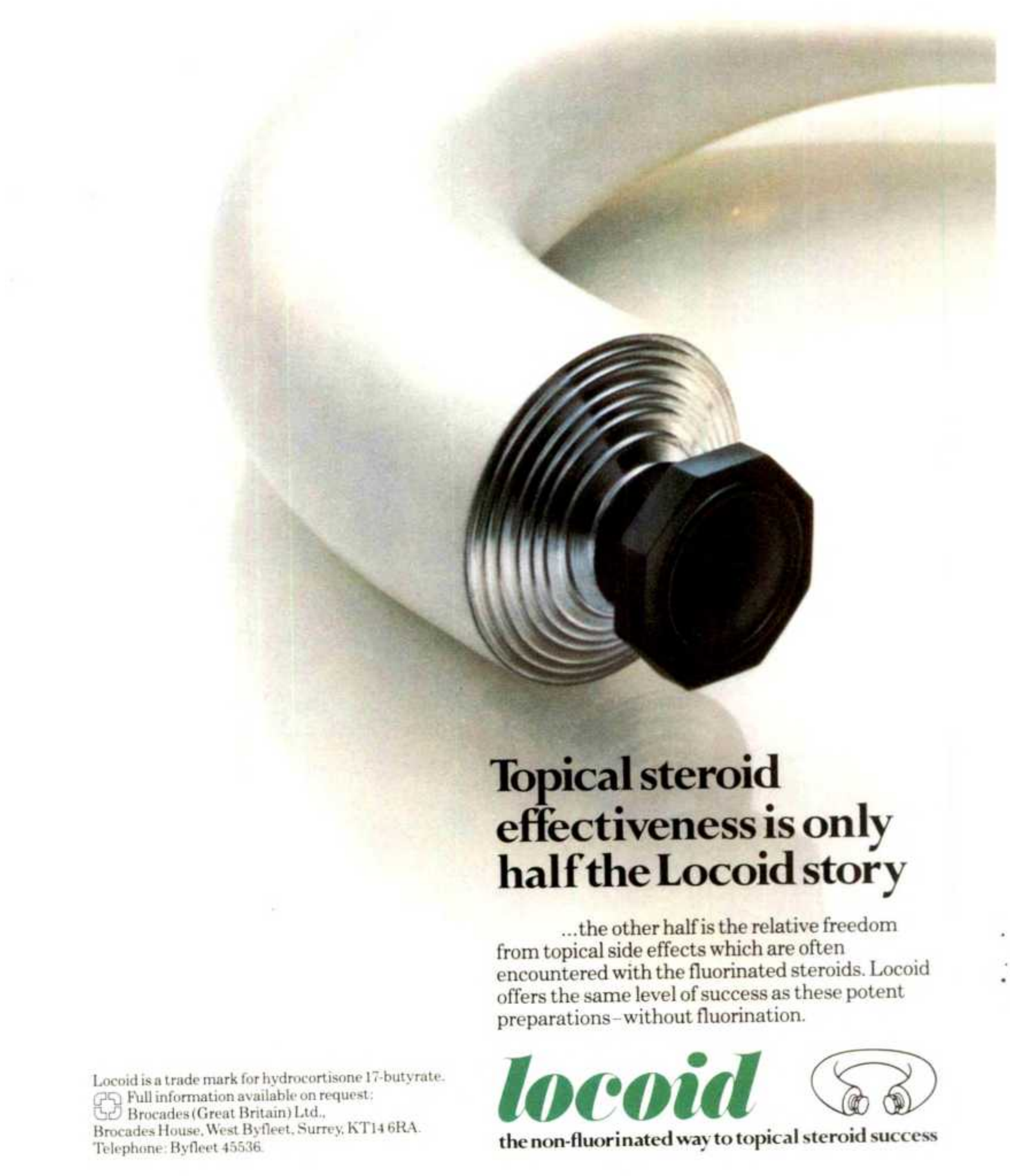

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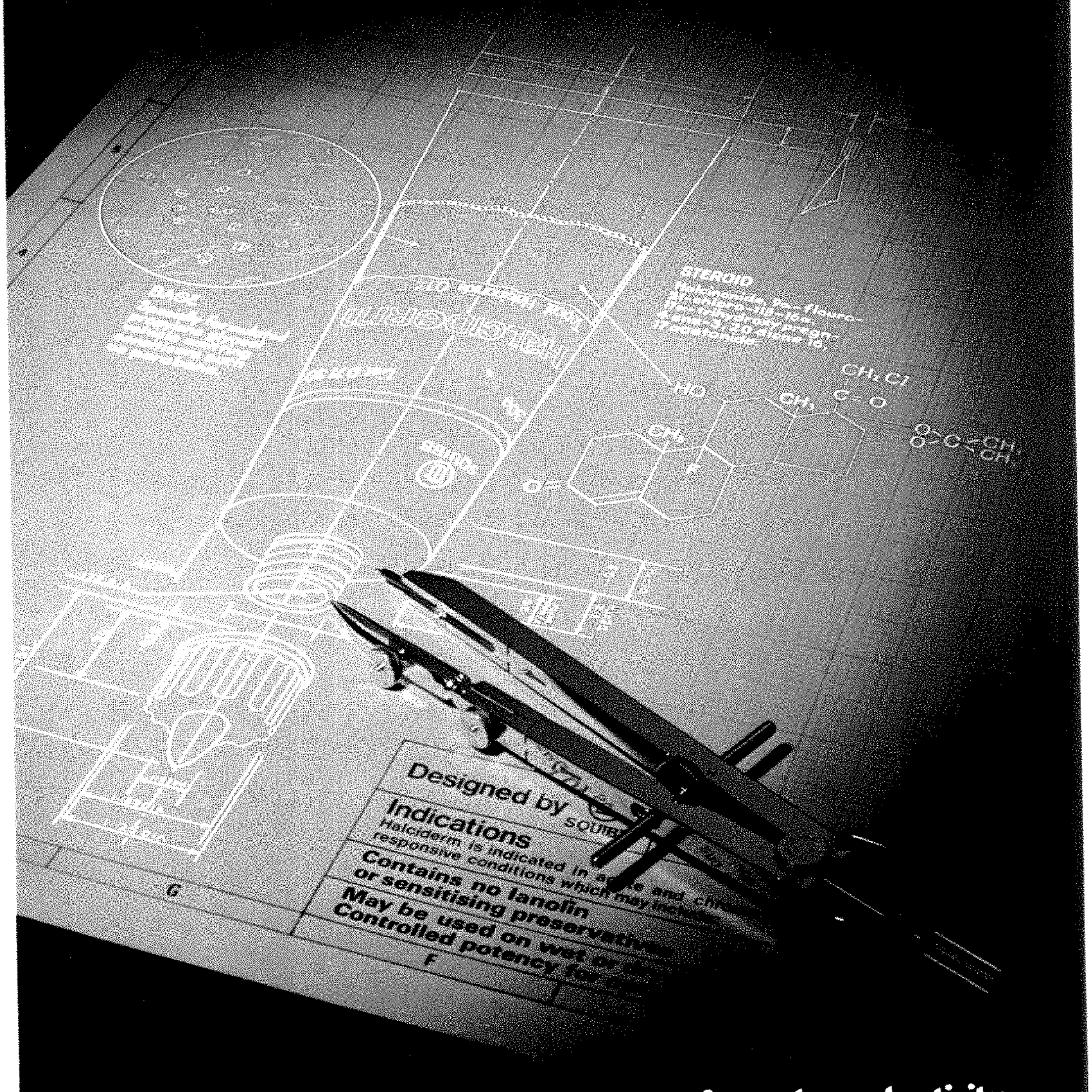

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Reference 1 Olansky, S.; in *Current Therapy*; edited by H F. Conn, London: Saunders, 1969, p.646.

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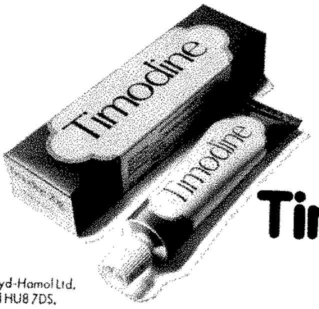

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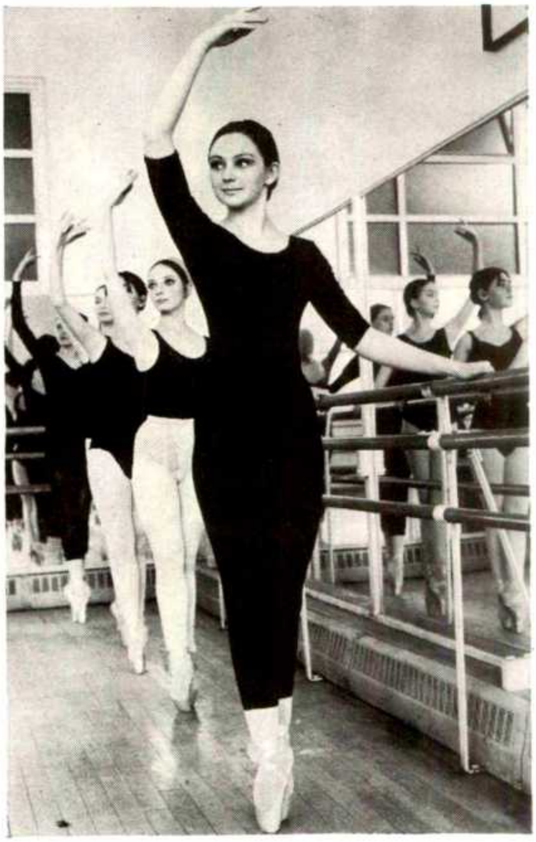

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1, 2, Practitioner 1970, 204, 639

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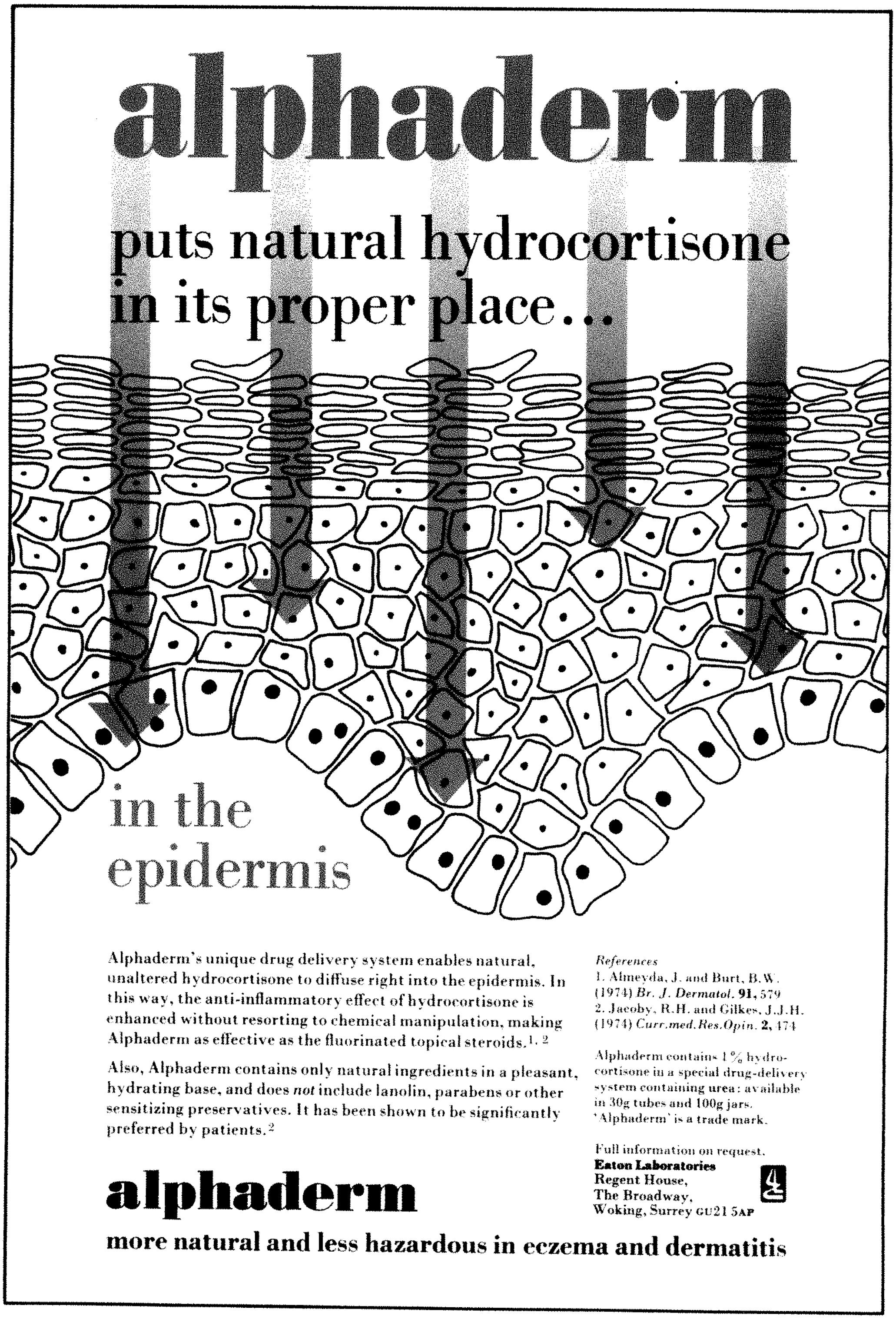

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1. Almeyda, J. and Burt, B.W. (1974) *Br. J. Dermatol.* **91**, 579
2. Jacoby, R.H. and Gilkes, J.J.H. (1974) *Curr.med.Res.Opin.* **2**, 474

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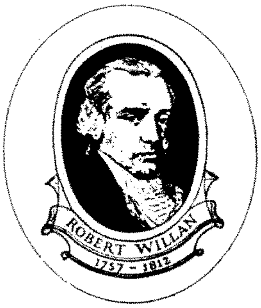

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Clinical and Laboratory Investigations

An animal model for screening drugs for antipsoriatic properties using hydroxyapatite to isolate DNA rapidly from the epidermis

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SUMMARY

A new, rapid, simple and accurate procedure for extracting DNA from the epidermis is described involving hydroxyapatite. This compound has an unique property of high affinity for double stranded native DNA, but none for RNA and protein. The technique has been applied to a hairless mouse model for screening systemic and topical agents for cell inhibitory properties in proliferating epidermis for potential use in the therapy of psoriasis.

Mitotic counts, autoradiography and a standard chloroform procedure for isolating DNA have confirmed the results obtained using hydroxyapatite column chromatography. In addition, compounds which are known to be effective in psoriasis, have been found to be effective in the system suggesting that it should be a good predictive screen for new therapies. Of several compounds screened for topical activity, cycloheximide has been found to be very effective in the system and preliminary clinical studies confirm its efficacy in psoriasis.

A hairless mouse screening model for new therapies for psoriasis has recently been described by our group for testing topical and systemic drugs for antiproliferative properties in the epidermis (du Vivier & Stoughton, 1975). The criteria for such activity were the reduction of the mitotic index and the inhibition of DNA synthesis as shown by a decrease in the incorporation of radioactive thymidine into DNA. The DNA was extracted using a standard chloroform deproteinization procedure and although allowing for a quicker and more accurate assessment of compounds than does processing tissues for mitotic counts, a considerably easier and more rapid technique of isolating DNA has now been applied to the model. It represents a considerable advance in that DNA may be extracted from twenty-four samples of epidermis by one technician in 1½ h. It is the first time, to our knowledge, that the technique has been applied to the skin. Crude cell lysate from hairless mouse epidermis labelled

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with tritiated thymidine is loaded onto a column containing hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ which has an unique property of high affinity for native double stranded DNA but not for RNA and proteins (Britten, Pavitch & Smith, 1970). The RNA and proteins are washed through the column with a low salt buffer whilst the DNA remains bound until eluted with a high salt buffer. The absorbance of the DNA at 260 nm is measured in a spectrophotometer from which the amount of DNA per ml of sample may be calculated and then the radioactivity of the DNA is determined. If a drug has been previously given to the animal it may be assumed to have antimetabolic properties in the epidermis if the specific activity of the DNA is significantly decreased.

MATERIALS AND METHODS

Experimental design of animal model

The dorsal skin of 3–4 month-old random-bred hairless mice (strain HRS/J) of either sex is irradiated using a germicidal lamp (General Electric G8T5) for 6 min at 20 cm. This causes increased mitosis, increased DNA synthesis, and epidermal hyperplasia between 48–72 h (Epstein, Fukuyama & Fye, 1970).

For assessment of topical applications 0·1 ml of the drug is applied to the neck of the animal at 1, 7, 24, 31 and 48 h after u.v. irradiation and the animal immobilized for 20 min to prevent removal of the test material. The posterior half of the animal (henceforth referred to as the back) is left untreated to monitor for any systemic effect of the agent. For assessment of systemic agents the drug is given at 48 h.

All animals are given 25 *μc* tritiated thymidine (Amersham/Searle), with a specific activity of 15·7 *c*/mmole, subcutaneously into the right thigh at 53 h and the animal is sacrificed by ether inhalation at 54 h.

The neck and back skin are removed separately (in the case of topical assays) or together (in systemic drug assays) and biopsies are taken for determination of the mitotic index (expressed as mitoses per 1000 interfollicular basal cells) and of the labelling index (number of basal cell nuclei containing more than 5 silver grains per 1000 interfollicular basal cells) for correlation with the results from the DNA extraction procedure.

Preparation of epidermis for DNA isolation

The epidermis is separated from the dermis by placing the skin dermis down on a plate heated to 55°C for 1 min. The epidermis can then easily be scraped away from the dermis with a scalpel blade. The epidermis is wrapped in aluminium foil, frozen in liquid nitrogen and stored at −20°C until used.

Cell lysis

Approximately 30 mg epidermis is taken and the cells are lysed in a tissue grinder in 5 ml of a solution containing 8 M urea, 1% sodium dodecyl sulphate, 10^{-3} M ethylenediaminetetraacetic acid and 0·24 M sodium phosphate buffer, pH 6·8.

Hydroxyapatite column chromatography

A 20 cm^3 glass syringe is used as a column. Tygon tubing with a clamp is attached to the nozzle. A filter (any colourless cotton material of suitable mesh) is placed at the bottom of the syringe on top of which is layered 1 cm of sand (Mallinckrodt 7062). The syringe is filled with 10 ml of a solution containing 8 M urea and 0·24 M sodium phosphate buffer, pH 6·8 (starting buffer) at room temperature. Crystals of Bio-gel hydroxyapatite (Bio-Rad 13596) are added and allowed to settle to form a bed about

1·5 cm deep. The crude epidermal cell lysate is added and washed through the hydroxyapatite with 90 ml of the starting buffer in 15 ml washes (after this volume there is no u.v.-absorbing material present indicating that all the RNA has been eluted). In order to achieve a flow rate of approximately 5 ml/min the surface of the hydroxyapatite should be gently disturbed with a glass hand pipette on one occasion after the lysate has been added. The urea is removed in 15 ml washes with 90 ml 0·14 M sodium phosphate buffer, pH 6·8. The hydroxyapatite is then ready for the elution of DNA. Six millilitres 0·48 M sodium phosphate buffer, pH 6·8 is added and the first 1 ml and subsequent three 1·5 ml are collected. Experience shows that the middle two samples contain the DNA. After use the hydroxyapatite is dissolved away by passing 5 N HCl through the syringe. The sand can be washed and used again. In addition we have shown that if necessary the hydroxyapatite may be reused. Any residual DNA is removed from the column by washing through with the 0·48 M phosphate buffer. This is checked by ascertaining that no u.v. light absorbing material is present by u.v. spectrophotometry and that no radioactivity is measurable. The 0·48 M buffer is washed out with the 0·24 M starting buffer and the column is ready for reuse. It should be noted that the sodium phosphate buffers are made up in equal parts of monobasic hydrous and dibasic anhydrous sodium phosphate.

Estimation of DNA content

The absorbance of DNA is measured in a spectrophotometer (Beckman Model 25) in quartz cuvettes using an u.v. light source at 260 nm. If the $^{A}260{:}^{A}280$ is less than 1·8 the sample is contaminated with protein indicating inadequate washing of the hydroxyapatite prior to the elution of DNA. This ratio is important for obtaining consistent and accurate results. Assuming that one optical density is approxi-

TABLE 1. Demonstrates the profound effect of systemic cyclophosphamide, topical chlorambucil and fluocinonide cream on mitosis and DNA synthesis in proliferating mouse epidermis

Drug	No. of animals	Site	Mitotic index	Labelling index	cpm/10 μg DNA	
					Hydroxyapatite technique	Chloroform technique
Saline	11	Back	63 ± 20	307 ± 64	1250 ± 420	1220 ± 550
Systemic cyclophosphamide	19	Back	15 ± 11	28 ± 26	140 ± 70	190 ± 140
M-pyrol	12	Neck*	52 ± 15	375 ± 94	1110 ± 260	N.D.
		Back†	62 ± 19	450 ± 78	1300 ± 280	N.D.
1% Chlorambucil	12	Neck*	6 ± 4	47 ± 34	270 ± 120	N.D.
		Back†	64 ± 9	505 ± 105	1190 ± 450	N.D.
Propylene glycol	16	Neck*	51 ± 13	N.D.	1350 ± 530	N.D.
		Back†	56 ± 13	N.D.	1470 ± 330	N.D.
Fluocinonide	10	Neck*	29 ± 17	N.D.	300 ± 100	N.D.
		Back†	69 ± 17	N.D.	1320 ± 430	N.D.

N.D., not done.

* Topically treated area.

† Untreated back skin of same animals used to monitor for a possible systemic effect of the topical agent.

Drugs. (a) 150 mgs/kg cyclophosphamide in saline v saline given intraperitoneally at 48 h after u.v.light. (b) 1% chlorambucil in methyl pyrrolidinone (M-pyrol) v M-pyrol alone applied topically at 1, 7, 24, 31 and 48 h after u.v.light. (c) 0·05% fluocinonide (Lidex) cream v propylene glycol applied at 48 h only after u.v.light.

Sacrifice. 54 h after u.v. light.

mately equal to 50 μg DNA/ml (Felsenfeld & Hischman, 1965), the DNA content of the sample may be calculated.

Preparation for liquid scintillation counting

One ml 16 M urea, to allow the Insta-gel to gel properly, and 4 ml Insta-gel (Packard) are added to 1 ml sample, briefly shaken and refrigerated for 30 min before counting. Results are expressed as cpm/10 μg DNA (the minimum yield of DNA was 10 μg/ml).

RESULTS

Two nitrogen mustard compounds were investigated to check the validity of the system as a screen for topical and systemic drugs with antiproliferative properties in the epidermis. Systemic cyclophosphamide significantly reduced DNA synthesis as determined by the hydroxyapatite technique. Similar results were obtained using a standard chloroform procedure for isolating DNA. Autoradiography confirmed the decreased DNA synthesis and the mitotic index was also reduced (Table 1). One per cent chlorambucil was investigated to determine whether an agent applied topically could be shown to interfere with DNA synthesis. The specific activity of DNA using the hydroxyapatite procedure was significantly decreased as was the labelling index. The mitotic index was also reduced (Table 1). These results confirmed that the hydroxyapatite procedure could be used in the investigation of antiproliferative drugs. In addition the effect of the chlorambucil is shown to be local and not systemic in that DNA synthesis and mitosis is only decreased in the neck area where the test agent was applied and not in the adjacent untreated back area.

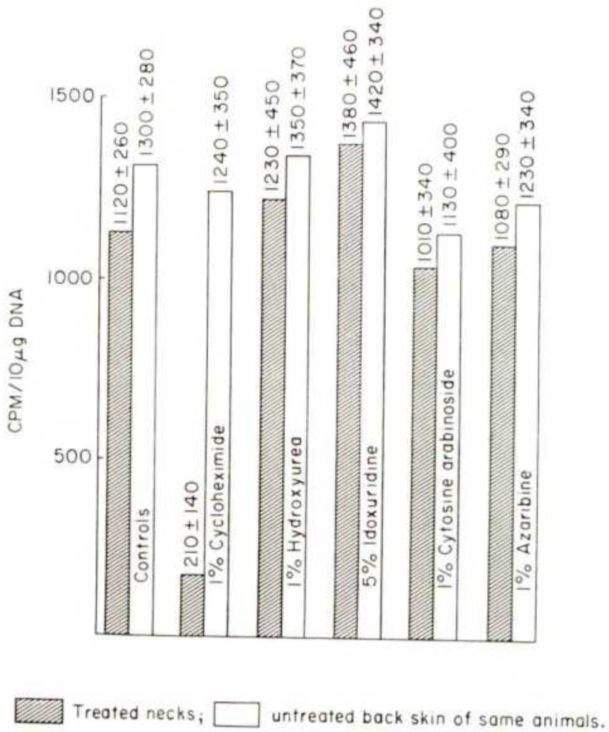

FIGURE 1. Drug Application: topically at 1, 7, 24, 31 and 48 h after u.v. light except cycloheximide (applied at 24, 31 and 48 h only). Sacrifice: 54 h after u.v. light. Number of animals: eight for each test agent. Results: 1% cycloheximide is an effective inhibitor of epidermal cell DNA synthesis.

A fluorinated corticosteroid, the most widely prescribed topical therapy for psoriasis, was assayed in the system and the results showed that DNA synthesis and mitosis is significantly reduced by fluocinonide cream (Table 1). One per cent cycloheximide (Actidione) in methyl pyrrolidinone topically reduces DNA synthesis significantly and suggested that this compound would be important to investigate clinically (Fig. 1). On the other hand, 5% idoxuridine, 1% hydroxyurea, 1% cytosine arabinoside, and 1% azaribine applied on five occasions in methyl pyrrolidinone did not significantly diminish DNA synthesis in comparison to methyl pyrrolidinone alone (Fig. 1).

DISCUSSION

Hydroxyapatite has the remarkable property of high affinity for DNA but not for RNA and proteins (Britten *et al.*, 1970), thus allowing DNA to be extracted very simply. Meinke, Goldstein & Hall (1974) have established the presence of RNA in the starting buffer elution using a $FeCl_3$-orcinol method (Schneider, 1945) and also of protein with a modification of the Folin phenol reagent method (Zak & Cohen, 1961). The diphenylamine reaction (Burton, 1956) for DNA is negative and no radioactivity is present in the sample either. On the other hand, the tests are negative for RNA and protein in the 0·48 M sodium phosphate buffer fraction but positive for DNA and the tritiated thymidine label is present. The melting profile characteristic of the DNA obtained with this technique is in close agreement (Meinke *et al.*, 1974) with the figure obtained for native mouse DNA using multiple chloroform extractions (Savitsky & Stand, 1966). Also, the guanine and cytosine base composition of the DNA calculated from the thermal denaturation temperature using the equation of Marmur & Doty (1962) is similar to that reported for mouse DNA (Savitsky & Stand, 1966; Kirby, 1959).

The advantages of this fractionation procedure are several. First, it is very rapid. Twenty-four samples of epidermis may be processed by one person in 4–5 h (this time includes lysing the cells, eluting the DNA, reading the absorbance in the spectrophotometer, and preparing the samples for liquid scintillation counting). The technique is also accurate since it requires the minimum of manipulation. Finally, it gives a high yield of DNA and so only small quantities of epidermis are required.

The technique has been applied to proliferating epidermis to test for agents with potential therapeutic value in psoriaris. Epstein *et al.* (1970) have shown that increased mitosis, DNA synthesis and epidermal hyperplasia occur 48–72 h after u.v. irradiation of hairless mouse epidermis, a fact which we have confirmed (du Vivier & Stoughton, 1975). Hairless mouse skin is particularly advantageous not only because of the ease of application of topical drugs but because it also resembles human skin in having similar penetration characteristics (Stoughton, 1974).

We have demonstrated that the hydroxyapatite purification method can substantially improve upon the laborious and time-consuming techniques of mitotic counts and autoradiography, since the results which we have derived from the new method parallel those we have obtained using mitotic counts, autoradiography and a standard DNA isolation procedure.

The results obtained from the model parallel those known for the treatment of psoriasis. Thus, in a previous publication (du Vivier & Stoughton, 1975) we have shown that 5-fluorouracil and nitrogen mustard topically are effective in the hairless mouse system as they are clinically (Nurse, 1963; Epstein & Ugel, 1970). Systemic cyclophosphamide is also effective in both situations (Higgins & Thompson, 1966) whereas topical cyclophosphamide is ineffective (du Vivier & Stoughton, 1975). Topical corticosteroids are the mainstay of the therapy of psoriasis and effectively inhibit DNA synthesis and mitosis in the model. Preliminary clinical studies show that topical chlorambucil clears psoriasis but unfortunately can cause contact dermatitis. On the other hand these studies have shown cycloheximide to be effective in psoriasis.

Cycloheximide is a glutarimide antibiotic, derived like streptomycin from *Streptomyces griseus*. Its primary site of action is inhibition of protein synthesis at some point past the formation of aminoacyl-S-RNA preventing the transfer of amino acids into protein. It is well known to interfere with DNA synthesis *in vivo* (Estenson & Baserga, 1966) and *in vitro*, including human epidermoid carcinoma cell culture lines, but it is not established whether this is independent of or secondary to the effect on protein synthesis (Bennett, Smithers & Ward, 1964). Further studies of its therapeutic efficacy in psoriasis are in progress.

Thus it would appear that the model should be a good predictive screen for potential therapeutic agents in psoriasis. In addition, the model provides information regarding the site of action of a topical agent, since the back skin of a treated animal acts as a control indicating whether a drug applied to the neck of the animal has acted locally or systemically. The results suggest that in the concentrations tested no systemic effect has occurred which is important in view of the cases of systemic toxicity which have been reported from topical agents (Peck, Guss & Key, 1972).

In summary a simple and accurate technique has been described for the rapid isolation of DNA from proliferating hairless mouse epidermis and a system established for screening drugs administered topically or systemically for potential therapeutic value in psoriasis.

REFERENCES

BENNETT, L.L., Jr, SMITHERS, D. & WARD, C.T. (1964) Inhibition of DNA synthesis in mammalian cells by actidione. *Biochimica et Biophysica Acta*, **87**, 60.

BRITTEN, R.J., PAVITCH, M. & SMITH, J. (1970) A new method for DNA purification. *Carnegie Institute Washington Yearbook*, **68**, 400.

BURTON, K. (1956) A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of desoxyribonucleic acid. *Biochemical Journal*, **62**, 315.

DU VIVIER, A. & STOUGHTON, R.B. (1975) An animal model of psoriasis and its use for screening topical and systemic agents for antiproliferative properties in the skin. *Journal of Investigative Dermatology*, **65**, 235.

EPSTEIN, J.H., FUKUYAMA, K. & FYE, K. (1970) Effects of ultraviolet radiation on the mitotic cycle and DNA, RNA and protein synthesis in mammalian epidermis *in vivo*. *Photochemistry and Photobiology*, **12**, 57.

EPSTEIN, E. & UGEL, A.R. (1970) Effects of topical mechlorethamine on skin lesions of psoriasis. *Archives of Dermatology*, **102**, 504.

ESTENSON, R.D. & BASERGA, R. (1966) Puromycin induced necrosis of crypt cells of the small intestine of the mouse. *Journal of Cellular Biology*, **30**, 13.

FELSENFELD, G. & HISCHMAN, S.Z. (1965) A neighbor-interaction analysis of hypochromism and spectra of DNA. *Journal of Molecular Biology*, **13**, 407.

HIGGINS, L.C., Jr. & THOMPSON, J.R. (1966) Psoriasis with arthritis: chemotherapy with cyclophosphamide nitrogen mustard and 6-mercaptopurine. *Southern Medical Journal*, **59**, 1191.

KIRBY, K.S. (1959) The preparation of deoxyribonucleic acids by the p-aminosalicylate-phenol method. *Biochimica et Biophysica Acta*, **36**, 117.

MARMUR, J. & DOTY, P. (1962) Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *Journal of Molecular Biology*, **5**, 109.

MEINKE, W., GOLDSTEIN, D.A. & HALL, M.R. (1974) Rapid isolation of mouse DNA from cells in tissue culture. *Analytical Biochemistry*, **58**, 82.

NURSE, D.S. (1963) Effect of antimetabolites on epidermal structures. *Archives of Dermatology*, **87**, 258.

PECK, G.L., GUSS, S.B. & KEY, D.J. (1972) Topical lomustine in the treatment of psoriasis. *Archives of Dermatology*, **106**, 172.

SAVITSKY, P.J. & STAND, F. (1966) A method for the preparation of mammalian deoxyribonucleic acid. *Biochimica et Biophysica Acta*, **114**, 419.

SCHNEIDER, W.C. (1945) Phosphorus compounds in animal tissues. I. Extraction and estimation of desoxypentose nucleic acid and of pentose nucleic acid. *Journal of Biological Chemistry*, **161**, 293.

STOUGHTON, R.B. (1974) Percutaneous absorption: a personal view. *Journal of Investigative Dermatology*, **63**, 305.

ZAK, B. & COHEN, J. (1961) Automatic analysis of tissue culture proteins with stable folin reagents, *Clinica Chimica Acta*, **6**, 665.

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Hair follicle kinetics in psoriasis

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SUMMARY

The rate of epidermal cell production has been measured in the various parts of scalp hair follicles in patients with psoriasis of the scalp, and in controls with normal scalp skin. An *in vitro* technique was used in which thin tissue slices were exposed to tritiated thymidine enabling cells about to divide to become autoradiographically labelled. Biopsies from thirteen patients with psoriasis and nine controls were examined in this way. The labelling indices of the interfollicular epidermis was 27·5% in the psoriatics and 9·5% in the controls. The upper part of the external root sheath had a labelling index of 28% in the psoriatic group compared to 13·9% for the controls. The labelling indices of the sebaceous glands, matrices and external root sheaths were very similar in the two groups. The findings support the hypothesis for a dermal stimulating influence causing the increased epidermopoiesis seen in psoriasis.

The increase in the rate of epidermal cell proliferation in psoriasis is well known. The heightened epidermopoiesis has been shown in a variety of ways. For example, Fisher & Wells (1968) found a three or four times increase in the mitotic index of psoriatic plaques using a colcemid induced mitotic arrest technique and Weinstein & Frost (1968) found an autoradiographic labelling index of 22·7% in the lesions of psoriasis after the intracutaneous injection of tritiated thymidine compared to 5·2% in normal skin. Similarly, Porter & Shuster (1968) found that there is an increased rate of transit of ^{14}C labelled amino acids through psoriatic epidermis and Marks (1975) has demonstrated an increase in the rate of incorporation of tritiated thymidine by keratotome slices taken from patches of psoriasis. The hair originates from an epidermal structure (the pilosebaceous follicle), the constituent cells of which do not differ fundamentally from the cells of the interfollicular epidermis and have the same embryonic origin. It would not be unreasonable to suggest that the rate of hair growth might be increased in psoriasis *pari passu* with the observed increased rate of epidermal cell production. However, this does not appear to be the case and Comaish (1969) using ^{35}S cystine demonstrated that the rate of hair growth in psoriasis was in fact normal. There are important implications contained in the observation of normal hair growth in psoriasis as far as the pathogenesis of the disease is concerned. In this investigation we have examined the rate of cell replication of the individual compartments of the pilosebaceous apparatus in the psoriatic scalp to determine whether any part of the hair follicle is involved.

PATIENTS AND METHODS

Thirteen patients with typical psoriasis of the scalp who had no treatment in the previous 3 weeks were investigated. There were 6 females and 7 males aged 22–65 years (mean 39·5 years). Nine patients (2 females, 7 males, aged 27–72 years, mean 46·8 years) with minor skin complaints not involving the scalp and no history of psoriasis were also examined. Punch biopsies (5 or 7 mm) were taken from all the patients and controls who had given their informed consent. The biopsies were cut into thin slices 1–2 mm thick using sharp, disposable scalpels (Gillette) and incubated in an orbital incubator (Gallenkamp) for $2\frac{1}{2}$ h in unsupplemented Eagle's minimal essential medium at 37°C at high humidity and in an atmosphere of 95% air and 5% CO_2. Tritiated thymidine (specific activity 20 Ci/mmole. Batch no. 43) was added to the medium in a concentration of 2·5 μCi/ml at the start of incubation. The skin slices were removed from the medium at the end of incubation, fixed in 10% formalin and processed by routine histological methods. Paraffin sections were 'deparaffinized' and dipped in nuclear emulsion (Ilford K.2). They were then exposed in dark boxes for 2- and 3-week periods prior to development in Kodak developer D.19. The developed sections were stained in haematoxylin and eosin and mounted.

Nuclei that had more than 6 grains above them over 'background' were counted as labelled cells. Only areas that were uniformly heavily labelled were assessed as there were areas in the centre of the tissue slices into which the thymidine had not diffused and where there was little or no labelling. Labelling indices were determined for the matrix and the external root sheath by expressing the number of labelled cells present as a function of the total number of cells in that compartment. The upper external root sheath was separately assessed (that part above the entry of the sebaceous duct). Labelling indices for sebaceous glands were determined by expressing the number of labelled cells as a

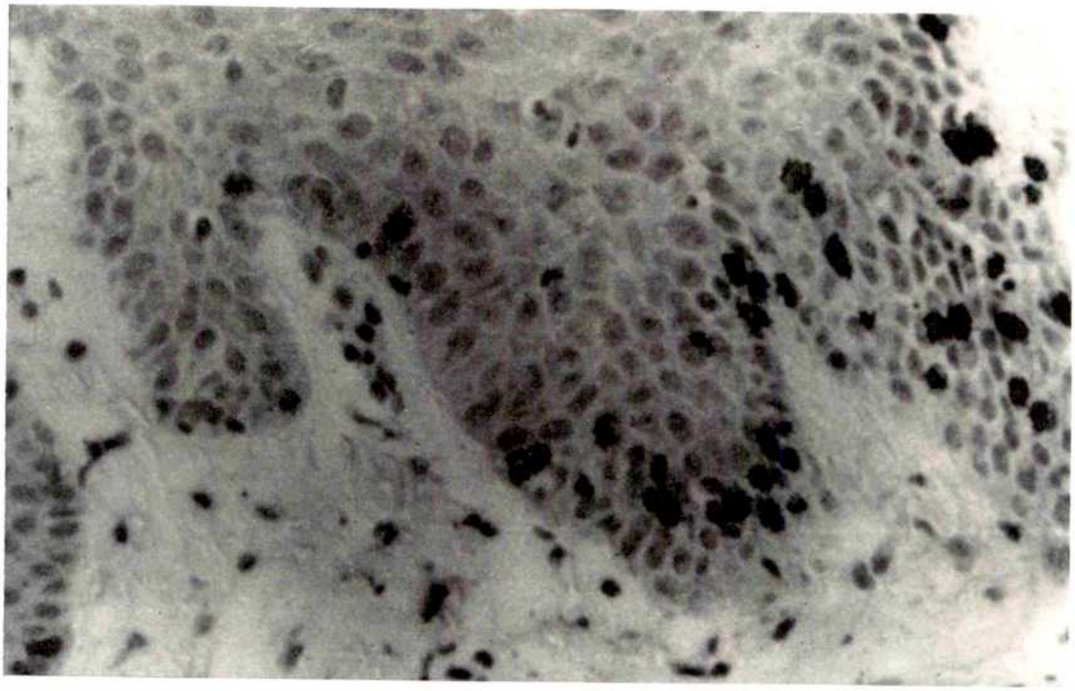

FIGURE 1. Interfollicular epidermis in biopsy from a patient with psoriasis of the scalp showing many autoradiographically labelled cells (H & E, × 150).

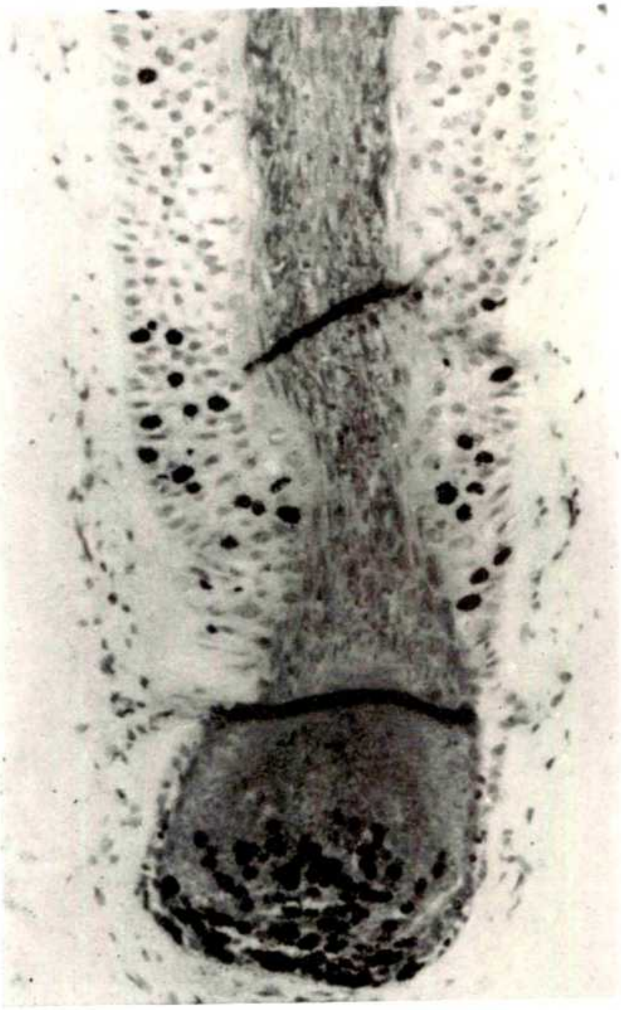

FIGURE 2. Hair follicle in section from scalp biopsy of patient with psoriasis. There are many labelled cells in the matrix and external root sheath (H & E, × 90).

function of the number of peripheral cells of the gland lobules. Labelling indices for the interfollicular epidermis were assessed by expressing the number of labelled basal and suprabasal cells as a percentage of the total number of basal cells. Between 400 and 2000 cells were counted for each 'labelling index'.

RESULTS

Typical labelling obtained in interfollicular epidermis, in a sebaceous gland, in a hair follicle matrix and in an external root sheath of biopsy material derived from patients with psoriasis is shown in Figs 1, 2 and 3 respectively. The labelling indices in the various parts of the hair follicles and in the interfollicular epidermis are given in Table 1.

There is a significant difference between the labelling indices for the interfollicular epidermis ($P<0.001$) and for the upper external root sheaths ($P<0.001$). The other epithelial compartment show no significant differences between control and psoriatic groups.

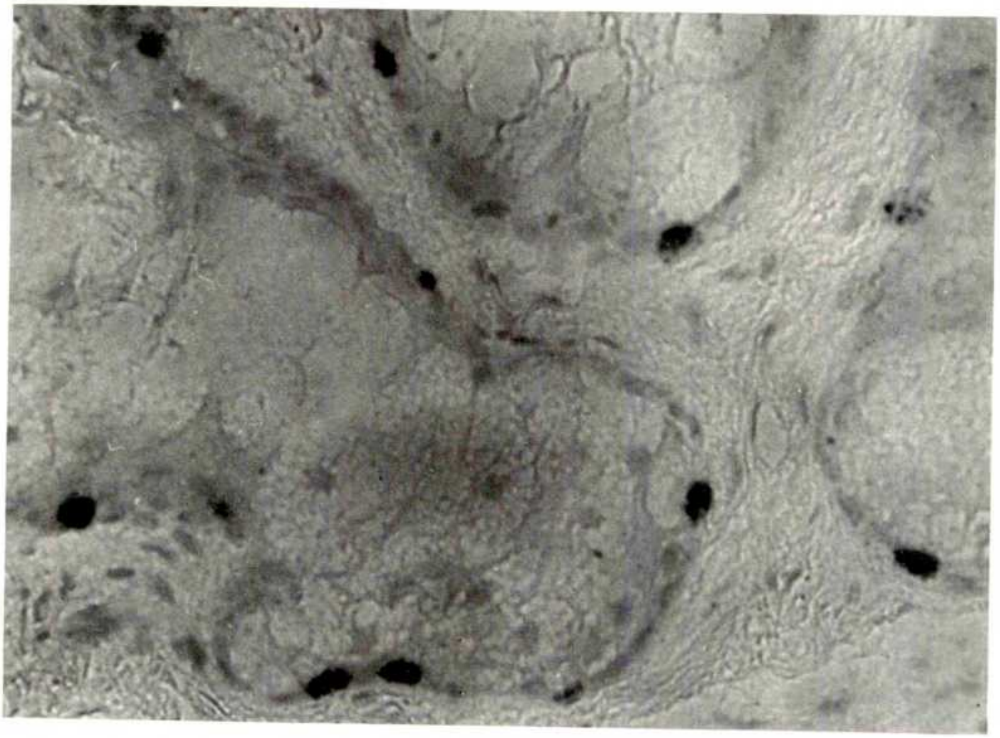

FIGURE 3. Sebaceous gland lobules showing labelled peripheral cells from a patient with psoriasis.

TABLE 1. Labelling indices (± s.d.) in interfollicular epidermis and epithelial compartments of scalp hair follicles in autoradiographs from biopsies from patients with psoriasis and controls

	Labelling Indices in hair follicle epithelia				
	Interfollicular epidermis	Sebaceous gland	Upper external root sheath	External root sheath	Matrix
Controls	9·5 ± 2·3	8·0 ± 1·8	13·9 ± 1·4	9·7 ± 1·9	35·8 ± 7·3
Psoriasis	27·4 ± 7·4	8·3 ± 1·3	28 ± 4·9	10·4 ± 1·2	36·1 ± 5·1

Hair follicles from scalps affected by psoriasis showed no particular morphological abnormality when compared to the follicles from the scalps of control patients. The interfollicular epidermis of the scalp when affected by psoriasis did not differ markedly in appearance from psoriasis elsewhere on the body.

DISCUSSION

The labelling index of 'normal' epidermis after the intracutaneous injection of tritiated thymidine and biopsy 50–60 min later is accepted by most laboratories as being in the region of 5%. In our hands, exposure to tritiated thymidine *in vitro* for periods of 2·5 h results in a labelling index of approximately 8% in epidermis derived from the limb or trunk. Our finding of a labelling index of 9·5% in normal control scalp epidermis using this technique may be explained by this area normally having an increased epidermopoiesis compared to limb and trunk skin. The figure of 27·5% for the affected interfollicular epidermis of the psoriatic scalp is approximately one-fifth more than that quoted by Weinstein & Frost (1968) but is low in relationship to our figures of 9·5% for the normal epidermis of the control subjects' scalps. However, the 2·9 times increase in labelling index that this represents for the psoriatic scalp interfollicular epidermis does not differ radically from the 2·3 times increase in thymidine incorporation by psoriatic plaques when compared to normal thigh epidermis (Marks, 1975). The similarity in labelling indices in the two groups for matrix epithelium, sebaceous glands and external root sheath is a not unexpected finding in view of the normal rate of hair growth and normal degree of greasiness of the skin in psoriatics observed clinically. The lack of an enhanced rate of cell replication in the bulk of the psoriatic's hair follicle and the increased epidermopoiesis in the upper external root sheath require some kind of interpretation. We find the following possible explanation attractive. If the drive to epidermopoiesis originated in the dermal papilla and was limited in its sphere of influence, then it may well only involve the interfollicular epidermis and the upper external root sheath (Fig. 4). It is certain that dermal stimuli can cause an accelerated rate of epidermal cell production (Schellander & Marks, 1973). The epidermal thickening seen over histiocytomata and other dermal lesions are other well-authenticated examples of there being a dermal mechanism which can influence epidermopoiesis. We find it difficult to accept that the increased rate of cell division in psoriasis is intrinsically epidermal when some epidermal structures are completely uninvolved. Although external influences may alter the various epidermally derived tissues, differentially (e.g. the steroid sex hormones) an intrinsic epidermal abnormality would most likely affect all epidermal compartments in some way. We believe that our findings of a normal rate of epidermal cell production in all the compartments of the hair follicle apparatus, except the upper external root sheath, pro-

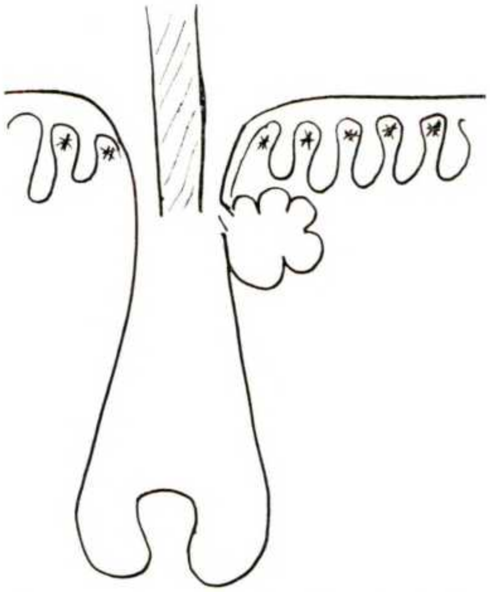

FIGURE 4. This diagram illustrates why the upper external root sheath might be involved in the psoriatic process. It is functionally very much in the field of influence of the papillary capillary.

vide some support for the suggestion that the cause of the epidermal hyperproliferation is dermal rather than epidermal.

ACKNOWLEDGMENT

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REFERENCES

COMAISH, S. (1969) Autoradiographic studies of hair growth in various dermatoses: Investigation of a possible circadian rhythm in human hair growth. *British Journal of Dermatology*, **81**, 283.

FISHER, L.B. & WELLS, G.C. (1968) The mitotic rate and duration in lesions of psoriasis and ichthyosis. *British Journal of Dermatology*, **80**, 235.

MARKS, R. (1975) Increased DNA and protein synthesis in the involved and uninvolved skin of patients with psoriasis. Paper read at British Association of Dermatologists meeting. July, 1975.

PORTER, D. & SHUSTER, S. (1968) Epidermal renewal and amino acids in psoriasis and pityriasis rubra pilaris. *Archives of Dermatology*, **98**, 339.

SCHELLANDER, F. & MARKS, R. (1973) The epidermal response to subepidermal inflammation. *British Journal of Dermatology*, **88**, 363.

WEINSTEIN, G.D. & FROST, P. (1968) Abnormal cell proliferation in psoriasis. *Journal of Investigative Dermatology* **50**, 254.

British Journal of Dermatology (1976) **94**, 13.

The repair of impaired epidermal barrier function in rats by the cutaneous application of linoleic acid

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SUMMARY

Epidermal barrier function in rats was experimentally impaired by two separate means, namely, by rendering the animals deficient in essential fatty acids and by evoking a primary cutaneous irritant response by treating with a solution of sodium laurate. Impaired barrier function was manifested by a greatly increased rate of transepidermal water loss. Application to the skin of sunflower seed oil, which is rich in linoleic acid, rapidly restored to normal the abnormally high rates of transepidermal water loss in both experimental cases, and it was shown with the essential fatty acid-deficient rats that there was a concomitant incorporation of linoleic acid of the sunflower seed oil into epidermal lipids. Cutaneous application of olive oil, which is low in linoleic acid but rich in the non-essential oleic acid, did not influence epidermal barrier function. A close relationship of barrier function and essential fatty acids is indicated.

INTRODUCTION

The skin is a barrier between the living organism and its environment, and this barrier function resides in the stratum corneum (Blank, 1965). As well as being a barrier to the percutaneous entry of materials into the body, the skin also regulates the loss of body fluids; indeed, the rate of transepidermal water loss is a convenient parameter for expressing barrier function. In some skin diseases, in which the normal architecture of the stratum is altered, impaired barrier function occurs, with, in particular, increased transepidermal water loss. For example, in psoriasis the rate of transepidermal water loss is greatly increased compared with that of normal skin (Grice & Bettley, 1967). Similarly in the experimentally induced condition of essential fatty acid deficiency in rats, which is characterized by several changes in epidermal structure (Basnayake & Sinclair, 1956; Menton, 1968), the rate of transepidermal water loss is abnormally high. Also primary irritation of animal skin may cause cutaneous water permeability to be increased (Grasso & Lansdown, 1972), and structural changes may also be seen in the epidermis (Prottey, Hartop & Ferguson, 1973). These examples emphasize the close relationship of normal barrier properties of the skin to normal structural integrity of the epidermis.

In essential fatty acid-deficient rats the abnormally high rate of transepidermal water loss may be lowered to normal levels by the re-introduction of linoleic acid to the diet, when the cutaneous lesions also disappear (Basnayake & Sinclair, 1956), suggesting a link between essential fatty acids and barrier function. We have recently shown (Press, Hartop & Prottey, 1974; Prottey, Hartop & Press, 1975) that essential fatty acid deficiency in man may be corrected by the cutaneous application of essential fatty acid (as sunflower seed oil, which contains 62% linoleic acid), which emphasizes the efficacy of the cutaneous route for introducing essential fatty acids into the body. In this paper, we have further examined the value of cutaneous application of sunflower seed oil, but in the context of repairing impaired barrier function. As models of increased cutaneous water diffusion we have chosen the EFA-deficient rat and the sodium laurate-irritated rat. The effect upon their impaired cutaneous barrier function by cutaneously applied sunflower seed oil is described.

MATERIALS AND METHODS

Treatment of essential fatty acid-deficient rats

Groups of male littermate rats of the Colworth-Wistar strain were weaned at 3 weeks onto a purified diet and an essential fatty acid-deficient diet. The purified diet consisted of 72·83% sucrose, 20·0% Fisons Genatosan (casein), 5·0% Jones-Foster salt mixture, 0·0468% water-soluble vitamins, 0·1% choline chloride and 2·17% sunflower seed oil. In the essential fatty acid-deficient diet the sunflower seed oil was replaced with 2·17% hardened coconut oil. The linoleic acid content of the sunflower seed oil was 62%, while the hardened coconut oil contained 2·0% unsaturated fatty acids but no essential fatty acids. Two groups of rats were fed each diet for 9 weeks and for 19 weeks respectively. EFA-deficiency was characterized by scaliness of the feet and tail, increased transepidermal water loss, and the appearance of 5, 8, 11-eicosatrienoic acid in the serum lipids, with the concomitant reduction of linoleic and arachidonic acids (Holman, 1968). By 9 weeks on the deficient diets the animals were all EFA-deficient. In the first experiment rats were studied at 8 and 18 weeks on the diet, in the second experiment, at 12 weeks.

In the first experiment, after 8 and 18 weeks on the control and experimental diets, a small area of approximately 1 cm^2 was clipped on the interscapular skin of each rat. The groups of control and EFA-deficient rats were each sub-divided into two groups, one of which had no cutaneous treatment while the other received 0·1 ml of sunflower seed oil to the clipped skin twice daily for 7 days. This was equivalent to approximately 120 mg linoleic acid per day.

In order to prevent ingestion of the oil, all the animals were fitted with a headpiece of rigid nylon around the neck without cutting into the skin. The thin conical walls of the neckpiece extended over the head of the rat. The open end allowed the rat to eat and drink but prevented it from licking its paws or any part of its back. During these 7 days the eyes, nose and penis of the rats were cleaned to avoid possible infection. Transepidermal water-loss measurements were made immediately before and after the 7 days' application of sunflower seed oil.

In the second experiment, animals were taken after 12 weeks on the deficient diet. The dorsal hair was removed by clipping, and then to one group of six animals 0·1 ml of a solution of 167 mg/ml sunflower seed oil in ether was applied to the shaved skin daily for 5 days. Assuming that sunflower seed oil contains about 60% by weight linoleic acid, this is equivalent to the application of 10 mg linoleic acid to the skin daily. To a second group of six animals a similar volume of an ether solution of 133 mg/ml olive oil was applied. Olive oil contains approximately 75% by weight oleic acid, thus the amount applied cutaneously per day was 10 mg oleic acid. A third group, but of normal rats fed on the control diet for 12 weeks, received 10 mg linoleic acid cutaneously, as above, to serve as controls to the EFA-deficient animals. In this second experiment the nylon head pieces were not fitted to the animals. Transepidermal water loss was measured daily immediately before application of oil for 6 successive days.

Treatment of rats with sodium laurate

Female weanling rats (approximately 50–80 g) fed water and spital diet *ad libitum* were used exclusively for this experiment. At the beginning of the experiment each animal was closely clipped dorsally with electric hair clippers.

The rate of transepidermal water loss was determined on the clipped area of each rat prior to any treatment. Each animal was washed with 0·25 M sodium laurate solution for a period of 30 min, as previously described (Prottey & Hartop, 1973). After a period of 24 h had elapsed, to allow the irritation response to develop, the water loss was determined again. Individual responses to treatment were varied, some animals exhibiting very high rates of water loss, whereas others had lower, yet still above normal, rates relative to control values. The animals were divided into three groups so that each group contained examples of both high and low rates of water loss after irritation. One group had 0·1 ml of undiluted sunflower seed oil rubbed onto the irritated area, a second group received 0·1 ml undiluted olive oil (the treated control group), and a third group were left untreated, serving as untreated controls to both treated groups. Oil was applied to the skin immediately after the second determination of water loss. The rate of transepidermal water loss was determined for a third time on each animal 24 h after receiving the oil.

Determination of transepidermal water loss

The method used was originally developed by Thiele & Schutter (1963); a stream of dry nitrogen was passed over the skin by means of a flow cell and its increased moisture content was measured with a Meeco electrolytic moisture analyser (Manufacturers Engineering and Equipment Corporation, Warrington, Pennsylvania, U.S.A.). The time required to make readings was approximately 30 min, during which the rats were immobilized with Nembutal (75 mg per kg intraperitoneally) and placed in a controlled temperature chamber at 34·5 ± 0·5 °C. The gas flow cell, which was fitted with a thermistor to measure skin temperature, was strapped to the clipped dorsal skin with Velcro tape (Selectus Ltd., Biddulph, Staffordshire) and transepidermal water loss and skin temperature were recorded simultaneously on a twin channel recorder before and after application of oil.

Extraction and analysis of epidermal lipids

Rat epidermis was separated by heat (Baumberger, Suntzeff & Cowdrey, 1942), the epidermal lipids purified by thin-layer chromatography and fatty acid compositions were determined by gas–liquid chromatography (Prottey, Hartop & Ferguson, 1972).

RESULTS

Transepidermal water loss in essential fatty acid-deficient rats

The increase in transepidermal water loss in animals maintained on an essential fatty acid-deficient diet is illustrated in Table 1. Rats on the control diet showed low rates of transepidermal water loss at 9 and 19 weeks, and the cutaneous application of sunflower seed oil for 1 week after 8 and 18 weeks respectively did not greatly alter these. Rats maintained on the deficient diet, however, showed a significant increase ($P = 0{\cdot}001$) in water diffusion compared with controls after 9 weeks; after 19 weeks the increase was greater still. After cutaneous application of sunflower seed oil for 7 days at 8 weeks there was a significant reduction in the transepidermal water loss of the EFA-deficient animals ($P = 0{\cdot}001$).

In the two deficient animals treated for 7 days, after 18 weeks there was also a marked reduction in water loss. However, in both treatment cases (that is, after 9 weeks and 19 weeks) the observed values

TABLE 1. EFA deficiency and transepidermal water loss

Diet	Treatment of skin	Transepidermal water loss (mg/cm²/h) after weeks on diet	
		9*	19†
Control	Nil	0·26 ± 0·09 (3)	0·24, 0·34
	SSO	0·20 ± 0·03 (3)	0·39, 0·30
EFA deficient	Nil	3·07 ± 0·90 (3)	4·36, 4·12
	SSO	1·34 ± 0·52 (3)	1·37, 1·58

EFA, essential fatty acid; SSO, sunflower seed oil.

* Results are mean data ± s.d. for number of animals in parentheses.

† Results are from individual animals.

of transepidermal water loss had not attained the very low level shown by control animals on a normal diet.

Epidermal lipid fatty acid composition in essential fatty acid-deficient rats

The application of sunflower seed oil to the skin of normal rats on the control diet for 9 weeks (Table 2) caused an increase in the proportion of linoleic acid in the free fatty acid fraction of the epidermal

TABLE 2. Distribution of fatty acids in separated lipids of epidermis of control and EFA-deficient rats at 9 weeks

Diet (9 weeks)	Cutaneous treatment at 8 weeks	Lipid fraction	Fatty acid (%)					
			16:1	18:1	18:2	20:3 (ω–9)	20:3 (ω–6)	20:4
Control	Nil	PL	7·0 ± 1·9	15·9 ± 1·9	9·4 ± 1·7	T	0·5 ± 0·9	6·2 ± 0·3
	SSO		6·6 ± 2·5	18·6 ± 1·0	12·6 ± 2·5	T	1·6 ± 2·5	5·4 ± 3·0
	Nil	SE	7·2 ± 1·7	15·2 ± 1·2	2·2 ± 1·0	T	0·4 ± 0·6	1·2 ± 0·0
	SSO		5·1 ± 1·3	14·2 ± 1·2	5·6 ± 0·6	T	0·3 ± 0·3	1·0 ± 0·6
	Nil	FFA	3·6 ± 0·8	14·3 ± 2·8	5·3 ± 1·6	T	1·2 ± 1·2	1·8 ± 0·7
	SSO		2·7 ± 0·2	18·8 ± 6·6	20·4 ± 15·8	0·3 ± 0·5	0·4 ± 0·7	1·7 ± 0·3
	Nil	TG	4·0 ± 0·9	12·6 ± 3·6	4·6 ± 1·5	T	0·6 ± 0·7	2·6 ± 1·9
	SSO		6·0 ± 2·4	14·8 ± 3·8	3·7 ± 1·5	T	1·1 ± 0·8	2·3 ± 2·4
EFA-deficient	Nil	PL	9·2 ± 4·6	25·7 ± 4·3	1·0 ± 1·2	4·1 ± 1·8	0·3 ± 0·2	1·2 ± 1·3
	SSO		8·3 ± 3·2	21·2 ± 4·4	8·4 ± 4·1	4·4 ± 2·3	1·0 ± 0·5	1·5 ± 0·9
	Nil	SE	8·3 ± 2·3	16·4 ± 4·3	0·5 ± 0·6	T	0·4 ± 0·4	1·8 ± 0·5
	SSO		7·3 ± 0·7	21·1 ± 2·0	1·3 ± 0·2	0·4 ± 0·7	0·6 ± 0·7	2·2 ± 2·1
	Nil	FFA	6·6 ± 2·1	16·7 ± 3·3	2·2 ± 1·3	2·1 ± 0·6	1·7 ± 0·8	2·0 ± 0·9
	SSO		4·5 ± 3·3	25·7 ± 5·7	22·2 ± 15·7	0·7 ± 0·6	0·8 ± 1·1	0·6 ± 1·0
	Nil	TG	7·4 ± 1·5	20·6 ± 11·7	3·0 ± 1·4	0·7 ± 1·1	0·6 ± 1·0	1·7 ± 1·3
	SSO		4·2 ± 1·8	20·4 ± 7·8	15·4 ± 6·9	2·3 ± 2·2	0·6 ± 1·0	1·6 ± 1·4

SSO, sunflower seed oil; PL, phospholipids; SE, sterol esters; FFA, free fatty acids; TG, triglycerides.
The results are the means of three animals ± s.d.
T, trace (< 0·1%).

TABLE 3. Distribution of fatty acids in separated lipids of epidermis of control and EFA-deficient rats at 19 weeks

Diet (19 weeks)	Topical treatment at 18 weeks	Lipid fraction	Fatty acid (%)					
			16:1	18:1	18:2	20:3 (ω–9)	20:3 (ω–6)	20:4
Control	Nil	PL	6·7, 7·9	14·6, 16·5	8·1, 10·2	T, T.	1·1, 2·2	5·8, 5·0
	SSO		5·8, 6·0	15·5, 16·1	10·8, 7·9	T, T	0·5, T	6·6, 3·5
	Nil	SE	5·4, 5·6	9·1, 15·6	2·4, 1·9	T, T	T, 2·8	2·7, 0·6
	SSO		5·6, 4·4	12·5, 14·4	2·2, 4·2	1·0, T	0·6, 5·1	1·5, 1·7
	Nil	FFA	2·9, 3·0	11·0, 14·3	4·5, 3·2	T, T	3·6, 0·6	8·8, 0·8
	SSO		4·0, 4·6	10·1, 19·9	6·8, 16·5	0·5, T	1·5, 4·2	2·9, 1·0
	Nil	TG	4·3, 4·0	18·5, 16·5	7·0, 7·3	T, T	T, 1·0	1·0, 0·9
	SSO		2·7, 4·4	18·9, 23·8	14·7, 8·1	T, T	T, T	1·7, 0·2
EFA-deficient	Nil	PL	10·5, 9·5	19·1, 19·6	0·9, 1·1	2·5, 11·4	T, T	0·9, 1·9
	SSO		9·1, 10·1	21·7, 24·2	5·9, 6·7	3·3, 3·4	T, T	1·2, 1·0
	Nil	SE	9·3, 7·8	21·4, 22·0	0·9, 0·9	0·3, 0·7	T, T	0·5, 1·0
	SSO		5·2, 4·2	17·8, 14·7	1·0, 3·2	T, 7·2	1·3, T	3·5, 1·1
	Nil	FFA	7·6, 8·0	22·2, 30·0	1·4, 2·8	4·9, 3·9	0·9, T	1·8, 2·2
	SSO		5·0, 4·2	17·0, 18·9	4·4, 12·9	1·5, 1·9	0·3, 1·1	2·3, 1·2
	Nil	TG	6·6, 9·9	16·1, 32·5	0·5, 3·5	T, 4·5	T, T	1·1, 3·6
	SSO		3·7, 2·7	22·7, 18·7	8·7, 7·6	0·2, 1·3	T, T	3·2, 2·0

SSO, sunflower seed oil; PL, phospholipids; SE, sterol esters; FFA, free fatty acids; TG, triglycerides.
The results are the individual values from two animals.
T, trace (< 0·1%).

lipids, but no marked changes were seen in the amounts of this in the other lipid fractions. Rats maintained on the essential fatty acid-deficient diet for 9 weeks (Table 2) showed increased oleic acid in the phospholipid and triglyceride fractions and in 5, 8, 11-eicosatrienoic acid (ω-9) in the phospholipids, together with a decrease in the linoleic and arachidonic acids of the phospholipids. The 5, 8,

TABLE 4. Comparison of the effects of cutaneously applied linoleic and oleic acid on lowering transepidermal water loss of EFA-deficient rats

Animals		Transepidermal water loss (mg/cm²/h)					
		Before application	After application (days)				
			1	2	3	4	5
EFA-deficient (6)	Linoleic acid (as sunflower seed oil)	2·33 ± 0·56 (12)	2·57 ± 0·42	2·64 ± 0·65	2·17 ± 0·70	1·41 ± 0·60	1·10 ± 0·38
EFA-deficient (6)	Oleic acid (as olive oil)		2·18 ± 0·43	2·22 ± 0·69	3·06 ± 1·31	2·76 ± 0·67	1·99 ± 0·78
Normal controls	Linoleic acid	0·48 ± 0·12 (6)	0·46 ± 0·10	0·48 ± 0·15	0·36 ± 0·15	0·42 ± 0·10	0·36 ± 0·18

Values in parentheses represent number of animals of each group, from which means and standard deviations were calculated.

11-eicosatrienoic acid, which is absent in normal tissues, is the biochemical 'hallmark' of essential fatty acid deficiency (Fulco & Meads, 1959). There was no appreciable change in the 8, 11, 14-eicosatrienoic acid (ω-6) of any of the lipid classes. When the deficient animals were treated with sunflower seed oil, however, the linoleic acid of the phospholipid fraction increased to virtually the control level, but no appreciable increase in the properties of the eicosatrienoic (ω-6) and arachidonic acids in any of the lipid fractions was seen after treatment. These latter two acids are metabolic derivatives of linoleic acid.

After 19 weeks on the control diet (Table 3) the fatty acid distribution of the epidermis was very similar to that of the 9 week controls, and the effect of cutaneous application of sunflower seed oil was to increase the proportion of linoleic acid in the free fatty acid and triglyceride fractions. After 19 weeks on the deficient diet (Table 3) the main changes were an increase in the proportions of oleic and 5, 8, 11-eicosatrienoic (ω-9) acids and a slightly less marked increase in palmitoleic acid in all lipid fractions, and the three essential fatty acids, linoleic; 8, 11, 14-eicosatrienoic (ω-6) and arachidonic acids were all low, compared to control animals. Application of sunflower seed oil for 1 week to the deficient rats at this time caused increases in linoleic acid in free fatty acids, phospholipids and triglycerides, but there were no changes in the 8, 11, 14-eicosatrienoic (ω-6) or arachidonic acids.

Rapidity of the response of lowered transepidermal water loss to cutaneous application

In a second experiment the effect of linoleic acid (as sunflower seed oil) on EFA-deficient rat skin during the first few days of treatment was investigated on a group of six animals. A separate group of six animals received olive oil (rich in oleic acid), and normal (non-deficient) rats treated with sunflower seed oil served as controls. Table 4 gives the calculated daily mean values of transepidermal water loss for the 3 groups. The standard deviations of these means can be seen to be quite large in some cases, indicating that there was a wide spread of individual values of transepidermal water loss within the various groups. In order to test the statistical significance of the results, the daily changes in transepidermal water loss as a result of oil application were examined by Student's '*t*'-test of the differences between means of the paired observations before treatment and day by day after treatment. When each mean daily observation of transepidermal water loss was compared with its corresponding pre-treatment value, with sunflower seed oil-treated EFA-deficient rats there was a marked reduction in transepidermal water loss after 4 days which continued into the 5th day; these reductions were significant at the $P = 0{\cdot}01$ level of significance. With the olive oil-treated animals, although the daily mean values of water loss fluctuated, none was significantly different from the pre-treatment values. Normal control animals lost very small amounts of water, which did not alter during 5 days of application of linoleic acid. In this experiment the animals received approximately 1/6th of the amount of linoleic acid administered to those of the first experiment. None the less, transepidermal water loss was markedly reduced by cutaneous application after 4 days.

Effect of sodium laurate on transepidermal water loss in normal rats

Prior to application of sodium laurate the rate of transepidermal water loss was determined in each of the eighteen rats, and the mean value was calculated as 0·096 mg/cm^2/h, with a standard deviation of ±0·047. These animals were weanlings (3–4 weeks old) and the mean value of water diffusion was much less than that seen with the control animals of Tables 1 and 4. As the latter were between 3 and 6 months of age, with different epidermal thickness and hair growth patterns, such differences are not unexpected.

In every case, 24 h after application of sodium laurate solution to the skin the rate of transepidermal water loss was seen to have increased. Most of the increased values were in the range 1–3 mg/cm^2/h, but three animals in particular had very high readings (the highest of which was 9·299 mg/cm^2/h)

TABLE 5. Effect of cutaneous application of sunflower seed oil and olive oil upon the recovery of transepidermal water loss after soap irritation

Treatment group	Mean water diffusion rate ± s.d. (mg/cm²/h)		
	Before irritation	After soap irritation*	After topical application
No application	0·091 ± 0·036 (6)	2·959 ± 3·201 (6)	1·777 ± 0·929 (6)
Sunflower seed oil	0·086 ± 0·051 (6)	2·411 ± 2·889 (6)	0·468 ± 0·155 (6)
Olive oil	0·110 ± 0·056 (6)	2·680 ± 2·595 (6)	2·279 ± 0·655 (6)

* Each animal was treated with 0·25 M sodium laurate for 30 min during 1 day.

The figure in parentheses denotes the number of experimental observations, from which the statistics were derived.

indicative of strong responses to sodium laurate application. The calculated mean increased value of transepidermal water loss for the eighteen animals was 2·68 mg/cm²/h, but, as the range of individual values was wide, the standard deviation of this mean was ±2·74 mg/cm²/h. None-the-less, a Student's '*t*'-test of the difference between the pre- and post-treatment means of paired observations was significant at $P = 0{\cdot}01$. The irritation response of the skin of these animals as a result of sodium laurate application was also varied, ranging from mild erythema to drying and cracking of the stratum corneum. No accurate subjective assessment of the degree of irritation was performed (Prottey, Hartop & Fergusson, 1973) and so semiquantitative comparison of transepidermal water loss and skin irritation could not be made.

Effect of cutaneous application of vegetable oils on water diffusion of sodium laurate-treated rats

After determination of the rate of transepidermal water loss in the irritated animals, they were divided into three groups, such that each group contained animals of high and low diffusion rates. Those in the first group received no further treatment, in the second group, sunflower seed oil, and in the third group olive oil. After a further 24 h water diffusion rates were measured and the results are shown in Table 5. All animals which received cutaneous sunflower seed oil exhibited lower values of transepidermal water loss (mean value of 0·408 mg/cm²/h), but a Student's paired '*t*'-test of the significance of these changes compared with after sodium laurate application alone proved to be non-significant at the 95% level of significance (presumably due to the wide spread of individual values, reflected by the standard deviations). In both the group of rats receiving olive oil and the untreated controls, the mean values of transepidermal water loss were not markedly lower than after sodium laurate application. In the group of rats receiving sunflower seed oil compared with both that group receiving olive oil and that group remaining untreated, transepidermal water loss was significantly lower ($P = 0{\cdot}01$), suggesting that it is the linoleic acid predominant in this oil which is responsible for repairing the abnormal barrier function.

DISCUSSION

We have shown that cutaneous application of linoleic acid, in the form of a triglyceride ester as sunflower seed oil, restores to normal the abnormally high rate of transepidermal water loss in two experimentally individual conditions of rat skin, namely, essential fatty acid deficiency and primary irritation by sodium laurate. Restoration to normal of high rates of water diffusion in essential fatty acid-deficient rats has been previously demonstrated by re-introduction to the diet of essential fat

(Basnayake & Sinclair, 1956). There are earlier references to the cutaneous application of linoleic acid to essential fatty acid-deficient rats: in 1936 Shepherd & Linn described how application of 'Vitamin F' (as linoleic acid was described) to the tail of an essential fatty acid-deficient rat cured the scaliness which characterized the condition; Grandel (1938) later extended these studies. We have recently demonstrated restoration of the cutaneous manifestations of EFA-deficiency in man by this means (Prottey *et al.*, 1975). Beneficial properties of cutaneously applied ethyl linoleate have also been shown recently by Jelenko, Wheeler & Scott (1972) for burned skin, in which the abnormally high rate of water transmission was reduced by up to 50%. Therefore, our finding here that impaired skin barrier function as a result of primary irritation may be restored by cutaneous sunflower seed oil is not unexpected.

As well as the similar results of sunflower seed oil application in both experimental models of impaired barrier function described here, there are also other similarities. It has been amply described (Williamson, 1941; Basnayake & Sinclair, 1956; Menton, 1968) that the skin in EFA-deficiency is hyperplastic and hyperkeratotic with a consequential thickening of the stratum corneum. We have shown (Prottey & Hartop, 1973; Prottey *et al.*, 1973) that rat skin treated with sodium laurate solution also exhibits morphological changes which have some similarities to essential fatty acid deficiency, such as hyperplasia, stratum corneum thickening and increased mitotic activity. Also, in EFA-deficiency in the rat, deranged lipid metabolism (Ziboh & Hsia, 1972) and accumulation of fat in the stratum corneum (Kingery & Kellum, 1965) have been reported. Similarly, it is known that following surfactant irritation of rat skin the epidermis synthesises more lipid (Prottey & Hartop, 1973), and lipid droplets may be observed in the stratum corneum (Tovell *et al.*, 1974).

Despite these similarities, it cannot be assumed that surfactant-irritated rat skin may be equated with detergent-treated skin. Elevation of the rate of transepidermal water loss is a general phenomenon that has been described in animals and man alike. Sweeney & Downing (1970) showed that solvent treatment of mouse skin increased the rate of water diffusion; tape stripped human skin has a similarly raised rate (Spruit & Malten, 1968; Eriksson & Lanke, 1971) and in certain skin diseases transepidermal water loss is increased (Grice & Bettley, 1967; Rajka, 1974). None the less, the beneficial action of sunflower seed oil in repairing barrier function may indicate an important function of linoleic acid; Onken & Moyer (1963) demonstrated the involvement of lipids in barrier function. Tables 2 and 3 indicate that cutaneous application of sunflower seed oil to EFA-deficient skin resulted in its incorporation into phospholipids, and we have similar (unreported) information for detergent-irritated skin so treated. Linoleic acid has been identified in human epidermal phospholipids (Vroman, Nemecek & Hsia, 1969).

It was found that the effect of sunflower seed oil on sodium laurate-irritated skin was rapid reduction in transepidermal water loss within 24 h, whereas on EFA-deficient skin a reduction in water loss of a similar magnitude required 4 to 5 days, and so the precise mechanism of the barrier repair may be different in these two instances. A physical occlusive effect may be discounted as olive oil did not effect such a reduction in water loss. Indeed, in separate studies (to be reported) we have examined other oils, and the beneficial action on impaired barrier function seems to be confined to certain polyunsaturated fatty acids of the ω-6 configuration; oils containing non-essential fatty acids have no effect.

Recently, Ziboh & Hsia (1972) have described how the cutaneous application of prostaglandin E2 to the skin of essential fatty acid-deficient rats cleared the scaly lesions, and also reversed the abnormal sterol ester metabolism. Thus it may be speculated that the repair of barrier function we have described above is, in fact, due to conversion of the cutaneously applied linoleic acid, via arachidonic acid, into prostaglandin E2, instead of (or in addition to) its incorporation into phospholipids which constitute the barrier function of the skin. This is currently under investigation.

REFERENCES

Basnayake, V. & Sinclair, H.M. (1956) The effect of deficiency of essential fatty acids upon the skin. In: *Biochemical Problems of Lipids* (Ed. by G. Popjak and E. LeBreton), p. 476. Butterworth Scientific Publications, London.

Baumberger, J.P., Suntzeff, V. & Cowdrey, G.V. (1942) Methods for the separation of epidermis from dermis and some physiological and chemical properties of isolated epidermis. *Journal of the National Cancer Institute*, **2**, 413.

Blank, I.H. (1965) Cutaneous barriers. *Journal of Investigative Dermatology*, **45**, 249.

Eriksson, G. & Lanke, L.O. (1971) Regeneration of human epidermal surface and water barrier function after stripping. *Acta dermato-venereologica*, **51**, 169.

Fulco, A.J. & Meads, J.F. (1959) Metabolism of essential fatty acids. VIII. The Origin of 5,8,11-eicosatrienoic acid in the fat-deficient rat. *Journal of Biological Chemistry*, **243**, 1411.

Grandel, F. (1938) Über Vitamin F. *Fette Seifen Anstrichmittel*, **45**, 150.

Grasso, P. & Lansdown, A.B.G. (1972) Methods of measuring, and factors affecting, percutaneous absorption. *Journal of the Society of Cosmetic Chemists*, **23**, 481.

Grice, K.A. & Bettley, F.R. (1967) Skin water loss and accidental hypothermia in psoriasis, ichthyosis and erythroderma. *British Medical Journal*, **iv**, 195.

Holman, R.T. (1968) Essential fatty acid deficiency. In: *Progress in the Chemistry of Fats and other Lipids* (Ed. by R.T. Holman), P. 279. Pergamon Press, Oxford.

Jelenko, C., Wheeler, M.L. & Scott, T.M. (1972) Studies in burns. *Journal of Trauma*, **12**, 974.

Kingery, F.A.J. & Kellum, R.E. (1965) Essential fatty acid deficiency. *Archives of Dermatology*, **91**, 272.

Menton, D.N. (1968) The effects of essential fatty acid deficiency on the skin of the mouse. *American Journal of Anatomy*, **122**, 337.

Onken, H.D. & Moyer, C.A. (1963) The water barrier in human epidermis. *Archives of Dermatology*, **87**, 90.

Press, M., Hartop, P.J. & Prottey, C. (1974) Correction of essential fatty acid deficiency in man by the cutaneous application of sunflower seed oil. *Lancet*, **i**, 597.

Prottey, C. & Hartop, P.J. (1973) Changes in glycerolipid metabolism in rat epidermis following exaggerated washing with soap solutions. *Journal of Investigative Dermatology*, **61**, 168.

Prottey, C., Hartop, P.J. & Ferguson, T.F.M. (1972) Lipid synthesis in rat skin. *British Journal of Dermatology*, **87**, 586.

Prottey, C., Hartop, P.J. & Ferguson, T.F.M. (1973) The effect of soap upon certain aspects of skin biochemistry. *Journal of the Society of Cosmetic Chemists*, **24**, 473.

Prottey, C., Hartop, P.J. & Press, M. (1975) Correction of the cutaneous manifestations of essential fatty acid deficiency in man by application of sunflower seed oil to the skin. *Journal of Investigative Dermatology*, **64**, 228.

Rajka, G. (1974) Transepidermal water loss on the hands in atopic dermatitis. *Archiv für Dermatologische Forschung*, **251**, 111.

Shepherd, M.I. & Linn, D.R. (1936) Evaluation of vitamin F. *Drug and Cosmetic Industry*, **38**, 629.

Thiele, F.A.J. & Schutter, K. (1963) Moisture meters for measuring the water balance of the human skin. *Proceedings of the Scientific Section of the Toilet Goods Association*, **40**, 20.

Tovell, P.W.A., Weaver, A.C., Hope, J. & Sprott, W.E. (1974) The action of sodium lauryl sulphate on rat skin—an ultrastructural study. *British Journal of Dermatology*, **90**, 501.

Williamson, R. (1941) A note on the epidermis of the rat on a fat-free diet. *Biochemical Journal*, **35**, 1003.

Vroman, H.E., Nemecek, R.A. & Hsia, S.L. (1969) Synthesis of lipids from acetate by human preputial and abdominal skin *in vitro*. *Journal of Lipid Research*, **10**, 507

Ziboh, V.A. & Hsia, S.L. (1972) Effects of prostaglandin E2 on rat skin: inhibition of sterol ester biosynthesis and clearing of scaly lesions in essential fatty acid deficiency. *Journal of Lipid Research*, **13**, 458.

Spruit, D. & Malton, K.E. (1968) Estimation of the injury of human skin by alkaline liquids. *Berufs-Dermatosen*, **16**, 35.

Sweeney, T.M. & Downing, D.T. (1970) The role of lipids in the epidermal barrier to water diffusion. *Journal of Investigative Dermatology*, **55**, 135.

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British Journal of Dermatology (1976) **94**, 23.

Elastotic Degeneration

A LIGHT AND ELECTRON MICROSCOPIC STUDY

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SUMMARY

The formation of elastotic material in the dermis of the exposed skin of elderly persons, and of two younger patients with manifestations of precocious degeneration, was studied by light and by electron-microscopy. Initially fine filaments or apparently granular material, or both, appeared around collagen fibres, which were mildly altered. On the exposed sites there was gradual loss of matrix and striation of the collagen forming an amorphous substance in which scattered, optically dense material appeared. Several appearances were common to all subjects.

There are numerous opinions on the origin of elastotic material (Tunbridge *et al.*, 1952; Teller, Vester & Pohl, 1957; Niebauer & Stockinger, 1965; Banfield & Brindley, 1963; Piérard, Kint & de Berasques, 1968; Braun-Falco, 1969). This may be because previous investigators have studied advanced examples of actinic degeneration. Earlier, and perhaps more subtle, changes are described in this work.

MATERIALS AND METHODS

There were two patients, aged 21 and 41 years, who exhibited precocious light-influenced degenerative changes. The skin of their necks was scleroatrophic as in a superficial chronic radiodermatitis and that of the trunk, limbs and gluteal regions, in decreasing degree, was dry with macular and mosaic-like superficial atrophy. Biopsies were obtained from the trunk and the gluteal regions in both patients and from the neck in the older.

Biopsies were also obtained from the back of the neck of three other men aged 50, 60 and 70 years, the first showing mild actinic degeneration and the third showing cutis rhomboidalis nuchae.

The following stains were used: haematoxylin and eosin (H & E), alcian blue, periodic acid Schiff (PAS), Gomori's reticulin stain, and von Kossa's stain for calcium salts. For electron microscopy the tissue was processed in the usual way, fixed in 1% OsO_4 buffered in veronal, embedded in epon, and contrasted with uranyl acetate and lead citrate.

RESULTS

Light microscopy: patients with precocious degeneration

Exposed skin. Underlying a narrow subepidermal band of oedematous collagen, a variable number of parallelly arranged fibres were present which stained positively with orcein-Giemsa stain, negatively with alcian blue, and violet with PAS. Elastic-stain positive globules were seen between altered collagen fibres.

Unexposed skin. The upper half of the dermis in all four specimens was oedematous, and the collagen bundles split. Throughout the dermis, often sparing a narrow subepidermal band, a considerable number of ovoid, spherical, elongated, or irregular masses, were present grouped or scattered, and measuring 12–25 μm (Fig. 1). Many of them stained black with orcein-Giemsa stain, black with Gomori's and were alcian blue and PAS negative. Others exhibited a spectrum of lighter to dark hues when stained with orcein-Giemsa, stained blue with Gomori's stain, lighter magenta to deep purple (positive) with PAS, and were alcian blue negative. In H & E sections, these globules as well as the upper dermis appeared hyalinized. A smaller number of less mature globules were seen in specimens from the gluteal region than in those from the trunk.

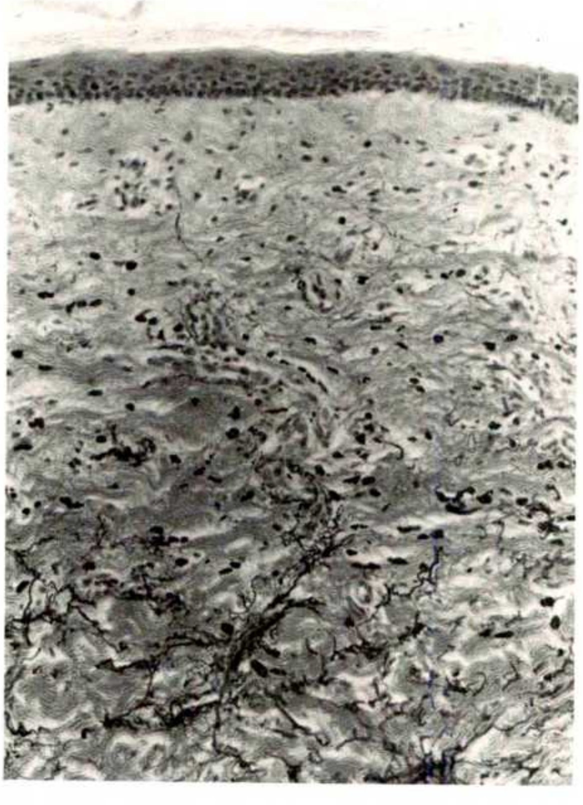

FIGURE 1. Throughout the upper dermis numerous orceinophilic globules are present. The elastic fibres are reduced. (The younger patient, skin of the trunk, acid orcein-Giemsa stain, × 87.)

Older patients with chronic actinic damage

Exposed skin (neck). Again there was a narrow subepidermal band of oedematous collagen. In the upper half of the dermis a variable number of thinner and thicker, often parallelly arranged fibres, staining positively with orcein-Giemsa stain, negatively with alcian blue, and violet with PAS, were present. In the two older persons, elastotic globules were present between the altered fibres. In the oldest person there were large irregular masses formed by thin, closely packed and randomly orientated fibrils. They stained moderately darkly with orcein-Giemsa, were PAS negative, and were basophilic in H & E sections.

Electron microscopy: patients with precocious degeneration

Exposed skin. Large 'fibres' and irregular masses of amorphous structure, often containing dense granules, were seen in the dermis. In some similar appearing fibres, striations could still be recognized. Their inner portion was translucent, and their membranes were often blurred and confluent. Optically denser material was sometimes present. Masses consisting of densely packed microfibrillar and/or granular material were rarely seen.

Unexposed skin (trunk and gluteal region). Within the upper dermis there were degenerative changes of varying intensity. The initial change consisted of thinly fibrillar and apparently granular material surrounding a segment of one to several fibres (Fig. 2a). Normal striation of such a segment could sometimes be recognized. Within such a group all fibres were not equally affected. The presence of optic-

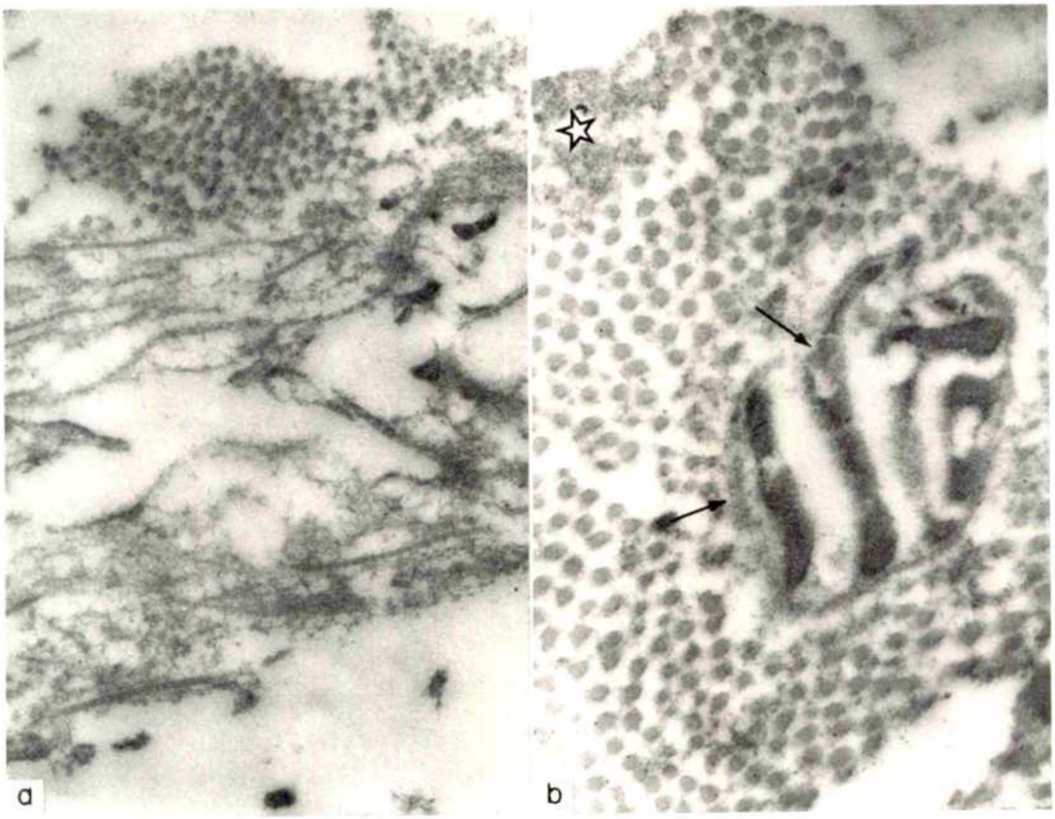

FIGURE 2(a). Longitudinal section through the central segment of an altered bundle. Rarefied fibres have lost their striation; fibrils are seen around them. (The older patient, skin of the trunk; × 24,000.) (b). Cross-section of a bundle. Central lying fibres are degenerate. Single oedematous fibres form the membrane-like structure within which some dense material is present. Fibrils and granular material are present at the periphery (asterisk). (The older patient, skin of the trunk; × 24,000.)

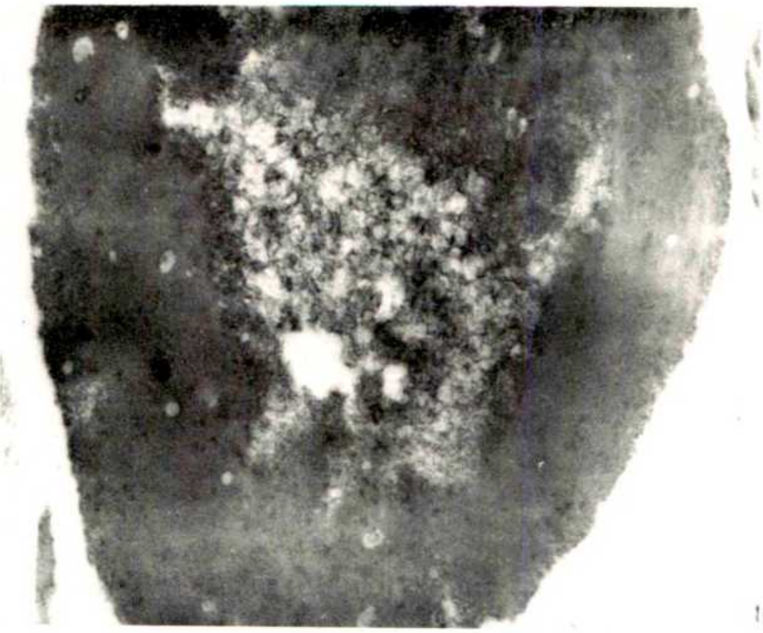

FIGURE 3. A segment of a bundle cross-sectioned. The outer region has changed into an amorphous mass, while fibrils are present nearer its centre. (The younger patient, skin of the trunk; × 16,000.)

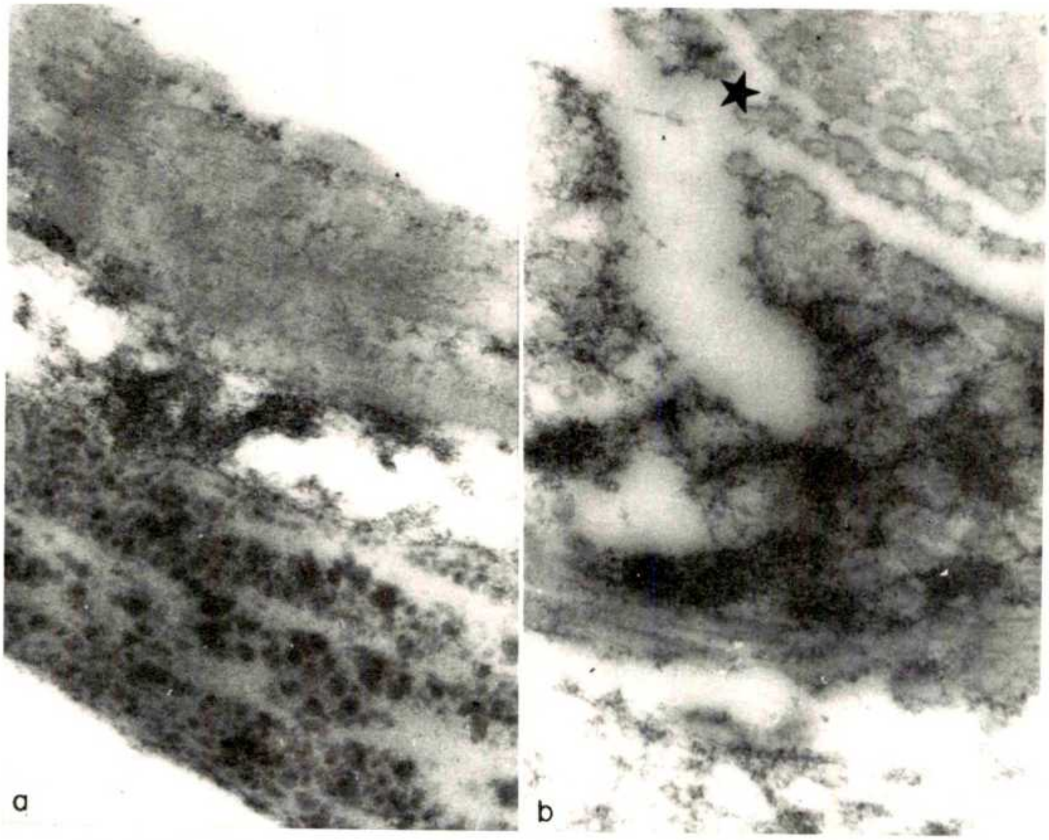

FIGURE 4(a). Longitudinal section through two 'elastotic fibres'. In one, striation can be recognized. Between fibres optically dense material is present. (Male, aged 60 years, neck; × 41,000.) (b). Cross-section of similarly affected fibres. The inner structure of the fibres has become lighter and the membranes partly confluent. A 'lace-like' pattern is also recognizable (asterisk). (Male, aged 60 years, neck; × 375,000.)

ally clear areas between such degenerating material suggested the disappearance of some material (Figs 2a & b), whereas increased density of some other areas suggested a more advanced stage in the degenerative process (Fig. 2b). Sections parallel to the long axis revealed that the centrally lying fibres were the first to degenerate. Frequently at a certain distance from the centre of a degenerating bundle a membrane-like structure was formed by partly degenerate and confluent fibres. Outside it fibres were also variably oedematous and optically lighter (Fig. 2b). In some bundles only the membrane-like structure with some inner and outer lying material remained. Some bundles developed into a more or less condensed fibrillar mass with only a few recognizable fibres. These and similar granular areas were sometimes replaced by amorphous material. For instance, a large degenerate bundle exhibited an amorphous periphery, while its central part was microfibrillar and granular (Fig. 3). In the more superficial part of the dermis, the altered bundles consisted mainly of closely packed fibrils surrounded by less altered fibres at the periphery. Such bundles were often surrounded by normal-looking fibroblasts.

Older patients with chronic actinic damage

Exposed skin. The findings (Figs 4a, b and 5) were virtually identical to those observed on the exposed skin in the older patient with precocious degeneration as described above.

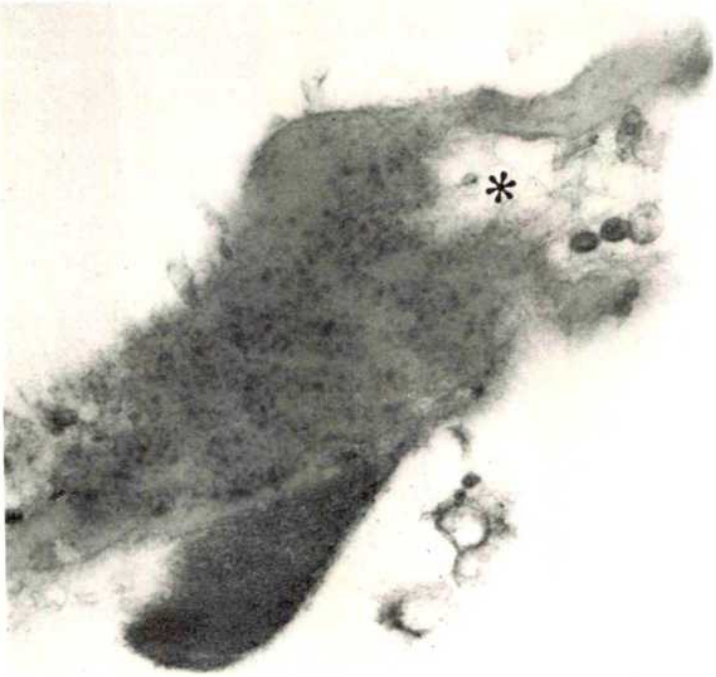

FIGURE 5. Side-by-side, fibrillar and amorphous stages of degeneration are present. Fibrils are also seen in the amorphous mass (asterisk). (Male, aged 60 years, neck; × 32,000).

DISCUSSION

Actinic elastosis has been explained on the basis of transformation of collagen fibres (Tunbridge *et al.*, 1952; Teller, Vester & Pohl, 1957; Niebauer & Stockinger, 1965), or of elastic fibres (Banfield &

Brindley, 1963; Piérard *et al.*, 1968), or of both with incorporation of a granular material (Mitchell, 1967). Braun-Falco (1969) felt the first step to be the appearance of homogeneous—possibly elastotic—material which led to a disordered arrangement within the collagen fibres, and to a disintegration of collagen fibrils which split into microfibrils. Elastotic fibres have also been found in skin of patients with chronic radiodermatitis (Ledoux-Corbusier & Achten, 1974). These findings permitted a study of the degenerative process from its onset to the final stage of elastotic degeneration. More advanced stages were common both to the unexposed skin in the patients with precocious degeneration and to the exposed neck of the elderly persons.

First of all either collagen fibres split into microfibrils and granular material, with subsequent appearance of the amorphous mass, or the latter is formed directly through the gradual loss of the matrix and membrane with subsequent confluence into future 'elastotic fibres'. At the amorphous stage, optically denser material appears. The formation of the amorphous material by the direct way seems to be more common in the exposed skin. Various stages of the degenerative process were seen side by side with normal looking collagen fibres. Cellular debris was often seen.

Before acquiring elastic stain properties, the degenerating material passes through a PAS positive phase. However, it is never alcian blue positive, though an increased amount of acid mucopolysaccharides can be seen in the upper dermis.

In the two patients with precocious degeneration there was an unusual number of fibroblasts seen around incompletely degenerated bundles. The fact that these bundles were more altered in their inner part may suggest an attempt at repair, presumably insufficient, rather than secretion of qualitatively altered collagen precursors.

The elastic fibres were also often altered. When amorphous, their smaller size was helpful in distinguishing them from similarly altered collagen bundles.

Elastotic changes such as 'a lace-like pattern, or multilobular holes' described as being of unknown origin (Ledoux-Corbusier & Achten, 1974) are most likely advanced stages of collagen degeneration. The denser material which appears towards the end of the process needs further elucidation.

Although at variance with the view of Ebner & Gebhard (1974) it would appear that 'elastic globes' are due to altered collagen rather than being new elastic fibres.

The clinical features in the patients could not be accurately classified into previously described light-influenced conditions. Some features were similar to those in 'hereditary sclerosing poikiloderma' (Weary *et al.*, 1969) a condition observed in black people and not influenced by sunlight. The patients and findings will be fully described separately (Stevanović 1975).

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REFERENCES

BANFIELD, W.G. & BRINDLEY, D.C. (1963) Preliminary observations on senile elastosis using the electron microscope. *Journal of Investigative Dermatology*, **41**, 9.

BRAUN-FALCO, O. (1969) Die Morphogenese der senil-aktinischen Elastose. Eine elektron-mikroskopische Untersuchung. *Archiv für klinische und experimentelle Dermatologie*, **235**, 138.

EBNER, H. & GEBHART, W. (1974) Elastic globes. Histochemische und elektronmikroskopische Untersuchungen. *Archiv Dermatologische Forschung*, **249**, 329.

LEDOUX-CORBUSIER, M. & ACHTEN, G. (1974) Elastosis in chronic radiodermatitis. *British Journal of Dermatology* **91**, 287.

MITCHELL, R.E. (1967) Chronic solar dermatosis: a light and electronmicroscopic study of the dermis. *Journal of Investigative Dermatology*, **48**, 203.

NIEBAUER, G. & STOCKINGER, L. (1965) Über die senile Elastosis. Histochemische und elektronmikroskopische Untersuchungen. *Archiv für klinische und experimentelle Dermatologie*, **221**, 122.

PIÉRARD, J., KINT, A. & DE BERASQUES, J. (1968) L'elastose senile. In: *Maladie du tissu elastique cutané. XII^e Congrès de l'Association de Dermatologistes et Syphiligraphes de Langue Française, Paris*, 1965, Vol. I, p. 373. Masson et Cie, Paris.

STEVANOVIĆ D. (1975) The diffuse and atrophic macular dermatosis. A premature and progressive light degenerative condition. *Dermatologische Monatschrift, To be published.*

TELLER, H.G., VESTER, G. & POHL, L. (1975) Elektronmikroskopische Untersuchungsergebnisse an der Interzellularsubstanz des Coriums bei Altersatrophie. *Zeitschrift für Haut und Geschlechtskrankheiten*, **22**, 67.

TUNBRIDGE, R.E., TATTERSALL, R.N., HALL, D.A., ASTBURY, W.T. & REED, R. (1952) The fibrous structure of normal and abnormal human skin. *Clinical Science*, **11**, 315.

WEARY, P.E., HSU, Y.T., RICHARDSON, D.R., CARAVATTI, C.M. & WOOD, B.T. (1969) Hereditary Sclerosing Poikiloderma. *Archives of Dermatology*, **100**, 413.

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The ultrastructural changes in the skin in dermatitis herpetiformis after withdrawal of dapsone

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SUMMARY

The development of skin lesions in patients with dermatitis herpetiformis after the withdrawal of their dapsone therapy was studied with the electron microscope.

In control biopsies from patients prior to cessation of treatment, membrane-bound vacuoles were found beneath the basal lamina of the epidermis as previously described. After dapsone withdrawal, there was an apparent increase in the number of vacuoles and occasionally several vacuoles appeared to have coalesced forming an early blister. At this stage, the basal lamina and associated hemidesmosomes were normal although in places there were small discontinuities in the basal lamina. Where the reaction was more intense, vacuoles and cells, mainly eosinophils, were embedded in fibrin deposits. Above this, the basal lamina was usually disrupted with involvement of the basal epidermal cells.

These results suggest that the vacuoles do play a part in the formation of the pathological lesion in dermatitis herpetiformis. In addition, the basal lamina is shown to be only secondarily involved. The nature of the vacuoles has still to be elucidated.

It has been shown that both the intestinal and cutaneous lesions in dermatitis herpetiformis (DH) can be improved by a gluten-free diet (GFD) (Fry *et al.*, 1969, 1973). However, the fact that dapsone improves the skin lesion but not the intestinal lesion, has delayed acceptance that gluten is implicated in the initial pathological reaction in both tissues. In a recent study of clinically normal skin in DH, membrane-bound vacuoles were found in the dermis below the epidermal basal lamina in patients taking dapsone, whereas they were not seen in patients controlled solely by a GFD (Riches *et al.*, 1973). From this evidence it was suggested that the vacuoles may be involved in an immunological reaction induced by the presence of gluten which has been put forward as a possible initiating factor in DH (Seah *et al.*, 1972). Therefore it would appear that dapsone merely suppresses the pathological process so that it does not become clinically manifest. In order to substantiate this hypothesis it is necessary to establish whether or not the vacuoles have a role in the formation of the typical blister. In this study, we have investigated the effects of dapsone withdrawal at the ultrastructural level in patients with DH.

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MATERIALS AND METHODS

Eight patients with DH whose rash was being controlled solely by dapsone were taken off their therapy and biopsies were obtained when erythema began to appear. Specimens were taken from the buttock by punch biopsy under 2% lignocaine anaesthesia and included uninvolved and erythematous areas. The tissue was fixed with 4% buffered glutaraldehyde and immediately post-fixed in 2% osmium tetroxide. After dehydration with a graded series of ethyl alcohols, the specimens were embedded in Taab resin. Thick sections were cut and stained with toluidine blue and examined to aid orientation of the tissue; subsequently thin sections were cut, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined on a Siemens Elmiskop I or an A.E.I. 801 S electron microscope.

RESULTS

Uninvolved skin. The appearances were similar to those observed in the uninvolved skin of patients taking dapsone (Riches *et al.*, 1973). In all patients, though not necessarily in all specimens, membrane-bound vacuoles were found in the dermis subjacent to the epidermal basal lamina (Fig. 1). In most cases the vacuoles occurred singly; some were associated with cell processes, but usually they were only surrounded by collagen fibres and ground substance. The vacuoles were not confined to the dermal papillae although elsewhere in the dermis they were less frequently found. Both the basal lamina and the associated hemidesmosomes appeared normal.

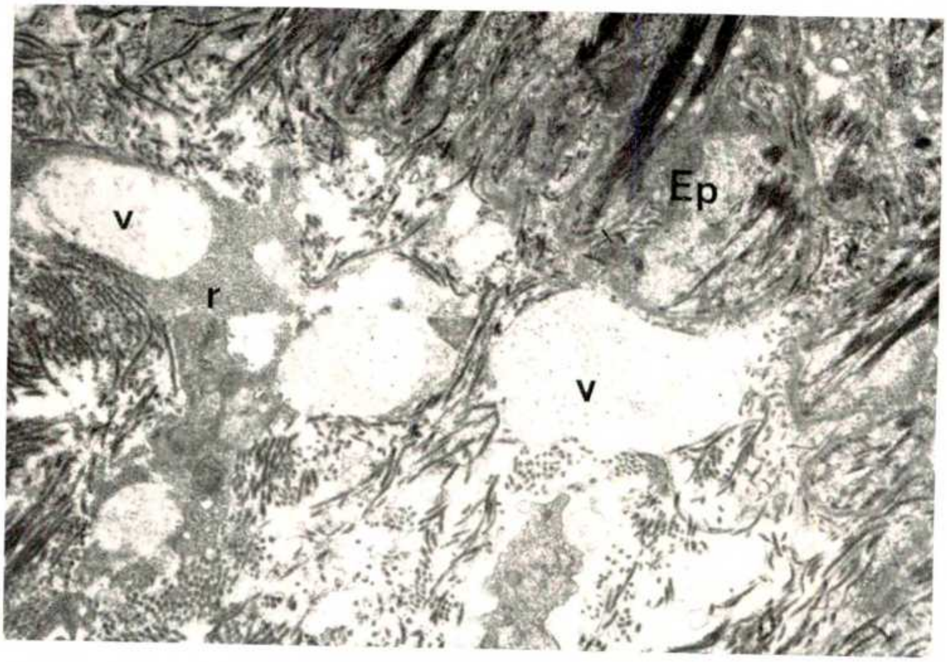

FIGURE 1. Uninvolved skin on dapsone therapy. Dermal–epidermal junction showing membrane-bound vacoules (v) and collections of reticular fibrils (r) in the dermis. Ep, epidermis (× 10,200).

Erythematous skin. The time taken for the erythematous reaction to appear after dapsone withdrawal varied from 2 to 7 days. In addition, the extent and intensity of the erythema differed between patients and consequently the ultrastructural appearances described were not necessarily found in any one specimen. However, the changes observed formed a pattern into which the reactions in each patient could be fitted.

In general, the dermis showed an increase in cellularity; some of the cells had definite characteristics of eosinophils, but many were more difficult to classify. As in the skin from 'normal' areas, membrane-bound vacuoles were found close to the basal lamina (Figs 2 and 3). However, they were frequently seen in groups of three or more and some of them seemed to be in the process of coalescing with a breakdown of the intervening membrane (Fig. 3). Collagen fibres with a normal pattern of striations were observed adjacent to the vacuoles, and where the membrane around the vacuole was absent, the collagen fibres were in direct contact with the material contained within the vacuole (Fig. 2). Between the collagen bundles, fewer reticular fibrils could be seen than in uninvolved skin and the ground substance resembled to some extent the fibrillar material contained within the vacuoles. At this stage, the basal epidermal cells were relatively normal and although, in places, the basal lamina was discontinuous, no thickening was seen and the hemidesmosomes were intact (Figs 2 and 3).

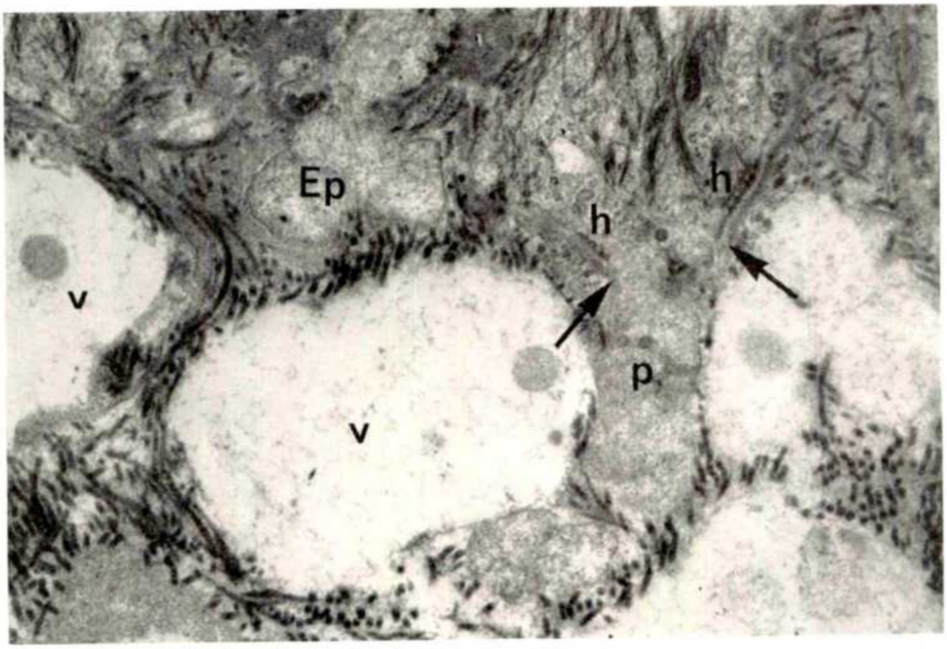

FIGURE 2. Erythematous skin after dapsone withdrawal. Membrane-bound vacoules (v) associated with a basal cell process (p) which projects through a discontinuity in the basal lamina (arrows). Note the adjacent normal hemidesmosomes (h). Ep, epidermis (× 20,000).

In areas where the reaction was more intense, fibrin deposits were found among which there were unclassified inflammatory cells, eosinophils and vacuoles (Fig. 4). Above these areas, the basal lamina was disrupted, but where it was present, normal hemidesmosomes were still observed. In addition to the dermal reaction, there was evidence of changes in the basal region of the epidermis. In particular,

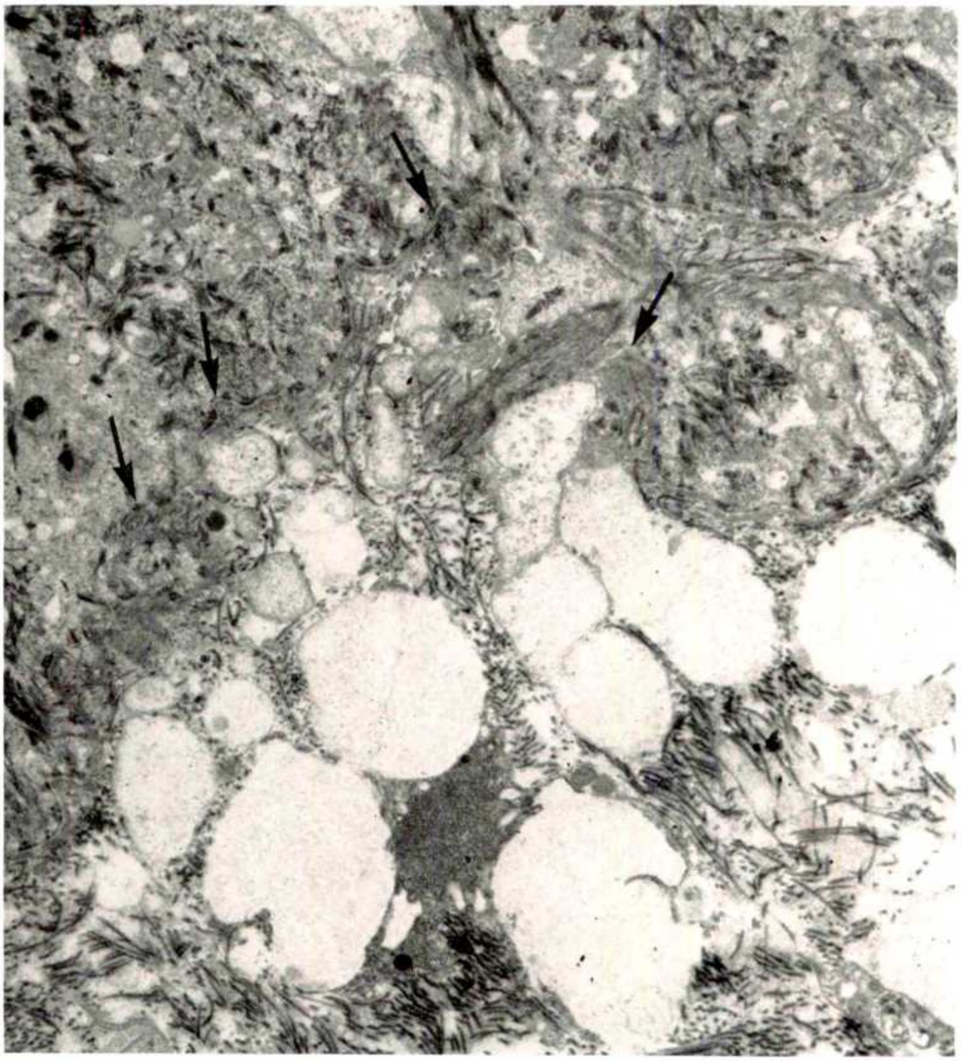

FIGURE 3. Erythematous skin after dapsone withdrawal. Numerous vacuoles, some confluent, in a dermal papilla. Two small discontinuities in the basal lamina (arrows) can be seen (× 9000).

there was a dilatation of the intercellular space which was filled with a material of low electron density similar in nature to that of the dermal vacuoles and sometimes this material was bound by an obvious membrane (Fig. 5). As the dermal inflammatory process became more intense, degenerative changes appeared within the basal cells which were now directly involved in the pathological reaction leading to the formation of the macroscopic blister.

DISCUSSION

The initial reactions which lead to blister formation in DH are still unknown although there is a gathering weight of evidence in favour of an immunological origin (Fry & Seah, 1974). Previous

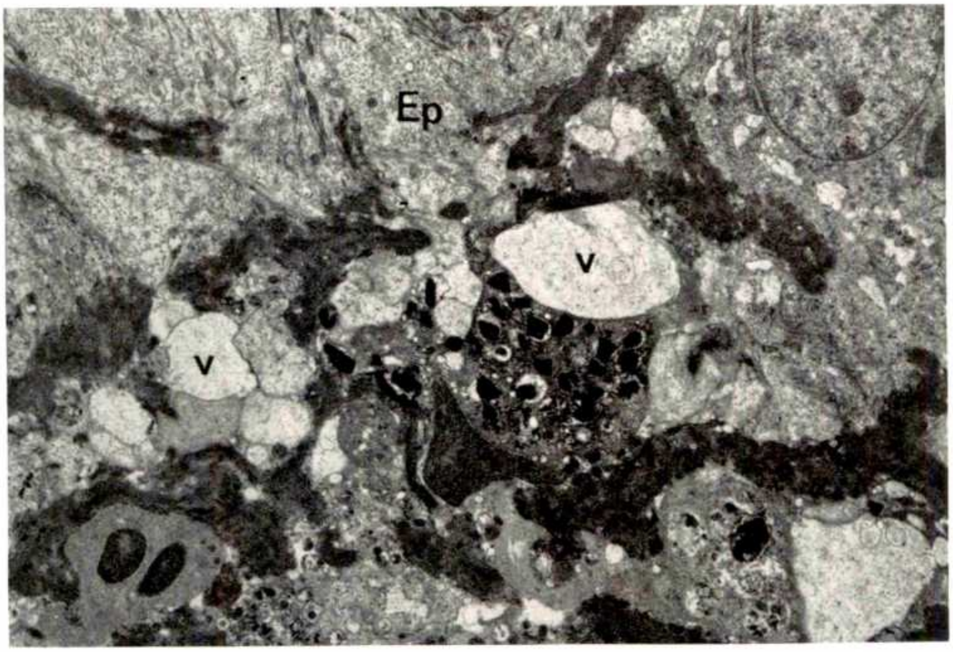

FIGURE 4. Erythematous skin after dapsone withdrawal. A region showing a more intense dermal reaction with vacuoles (v) and dermal cells embedded in fibrin deposits. The epidermis (Ep) is still relatively unaffected (× 4100).

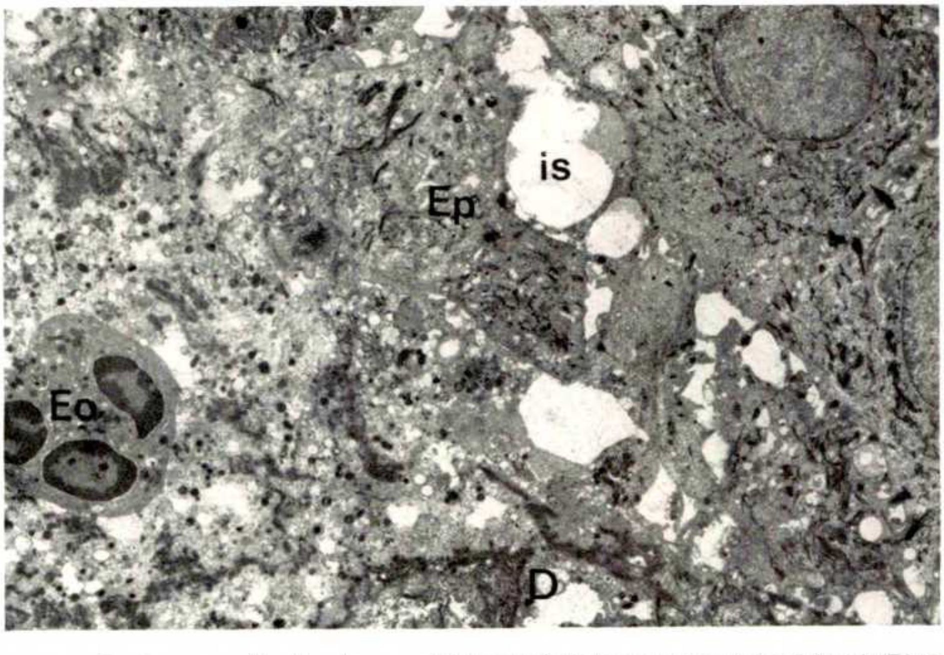

FIGURE 5. Erythematous skin after dapsone withdrawal. Both the epidermis (Ep) and dermis (D) are involved in the pathological process with disruption of the basal lamina. The intercellular space (is) between basal cells is dilated and filled with a fibrillar material. Note the infiltration of the epidermis by an eosinophil (Eo) (× 4100).

studies have suggested several sites where the lesion may originate ranging from the basal epidermal cells (Fry & Johnson, 1969), the basal lamina and hemidesmosomes (Jakubowicz & Dabrowski, 1971; Rodrigo, 1972) and the dermis immediately subjacent to the basal lamina (Bellone & Caputo, 1966; Pierard, Kint & Geerts, 1971). One reason why there is a lack of uniformity in results could be that different stages of development of the lesions were studied. In our own investigations, there were differences between patients in the time taken for the development of the erythema and this produced a similar variation in the appearance of the erythematous skin at the ultrastructural level. However, by examining a number of blocks from each specimen, some uniformity was achieved.

The vacuoles seen in the dermis of uninvolved skin of patients taking dapsone increased in number after treatment had been withdrawn. The grouping and coalescence of the vacuoles could lead to the formation of the definitive bulla. At this stage the non-specific reactions of fibrin deposition and accumulation of inflammatory cells become obvious thus masking the initial events. These findings, supported by previous evidence (Riches *et al.*, 1973), suggest that dapsone therapy merely suppresses the terminal stages of the pathological processes in DH and prevents the vacuoles coalescing to form a blister.

Pierard *et al.* (1971) described degeneration of the collagen fibres in early lesions of DH and suggested that this may be part of the initial reaction. In our studies, collagen fibres appeared relatively normal in so far as their striations were still visible, even in close proximity to the vacuoles. Further, if the vacuoles are formed as a result of the liquefaction of collagen, this process must be extremely localized. Rodrigo & Pereira (1973) put forward the view that the small fibrils may represent an arrest in development of elastic fibres, but in our studies there was very little evidence to support this theory. Recently it has been established that there is a non-collagenous component of connective tissue which reacts antigenically with anti-reticulin antibody (Pras *et al.*, 1974) and this antibody is found in the sera of some patients with DH (Seah *et al.*, 1973). Therefore, our original hypothesis that the vacuoles represent the products of an antigen–antibody reaction involving reticular fibres (Riches *et al.*, 1973) is still a possible explanation. Immuno-electron microscopic studies are in progress and these may help to elucidate the problem.

There is also the question as to whether or not the basal lamina is involved in the primary reaction. Our results indicate that the basal lamina only becomes disrupted during the later stages in the development of the blister. Up to the point at which the vacuoles coalesce and break down, the basal lamina is relatively intact and normal hemidesmosomes are present. Therefore, the findings presented in this paper again support the dermal origin of the lesion in DH and the basal lamina and basal epidermal cells are secondarily involved.

REFERENCES

BELLONE, A.G. & CAPUTO, R. (1966) Aspetti ultrastrutturali della dermatite erpetiforme di Duhring. *Giornale italiano di dermatologia*, **107**, 173.

FRY, L. & JOHNSON, F.R. (1969) Electron microscopic study of dermatitis herpetiformis. *British Journal of Dermatology*, **81**, 44.

FRY, L., MCMINN, R.M.H., COWAN, J.D. & HOFFBRAND, A.V. (1969) Gluten-free diet and reintroduction of gluten in dermatitis herpetiformis. *Archives of Dermatology*, **100**, 129.

FRY, L. & SEAH, P.P. (1974) Dermatitis herpetiformis. In: *Immunological Aspects of Skin Diseases* (Ed. by L. Fry and P. P. Seah), p. 22. Medical and Technical Publishing, Lancaster.

FRY, L., SEAH, P.P., RICHES, D.J. & HOFFBRAND, A.V. (1973) Clearance of skin lesions in dermatitis herpetiformis after gluten withdrawal. *Lancet*, **i**, 288.

JAKUBOWICZ, K. & DABROWSKI, J. (1971) The ultrastructure of the bullous lesions in Duhring's disease. *Polish Medical Journal*, **10**, 764.

PIERARD, J., KINT, A. & GEERTS, M.L. (1971) La forme pemphigoide de la maladie de Duhring. *Bulletin de la Société Française de Dermatologie et de Syphiligraphie*, **78**, 329.

Pras, M., Johnson, G.D., Holborow, E.J. & Glynn, L.E. (1974) Antigenic properties of a non-collagenous reticulin component of normal connective tissue. *Immunology*, **27**, 469.

Reynolds, E.S. (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology*, **17**, 208.

Riches, D.J., Martin, B.G.H., Seah, P.P. & Fry, L. (1973) Ultrastructural observations on uninvolved skin in dermatitis herpetiformis. *British Journal of Dermatology*, **88**, 323.

Rodrigo, F.G. (1972) Ultrastructural aspects of dermatitis herpetiformis (Duhring's disease). *British Journal of Dermatology*, **86**, 348.

Rodrigo, F.G. & Pereira, G.C. (1973) Ultrastructural observations on uninvolved skin in dermatitis herpetiformis. 'Elastic' versus 'reticular' fibrils. *British Journal of Dermatology*, **89**, 543.

Seah, P.P., Fry, L., Holborow, E.J., Rossiter, M.A., Doe, W.F., Magalhaes, A.F. & Hoffbrand, A.V. (1973) Antireticulin antibody: incidence and diagnostic significance. *Gut*, **14**, 311.

Seah, P.P., Fry, L., Stewart, J.S., Chapman, B.L., Hoffbrand, A.V. & Holborow, E.J. (1972) Immunoglobulins in the skin in dermatitis herpetiformis and coeliac disease. *Lancet*, **i**, 611.

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Centrofacial lentiginosis

A SURVEY OF 40 CASES

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SUMMARY

Forty patients with centrofacial lentiginosis have been studied. A greater than normal number of bone abnormalities, malformations due to dysraphia, endocrine dysfunctions, and neurological diseases, but not psychiatric impairment, were found.

Centrofacial lentiginosis was described by Touraine (1941a & b, 1958), who considered it to be a consequence of skull-base dysraphia and to be related to nerve damage and pigmentation disorders. It is characterized by the following:

(1) Lentigines, numerous on the nose and cheeks in a 'butterfly-like' pattern (Fig. 1), and sometimes also on the forehead, eyelids and upper lip. They are seen in the 1st year of life and continue to appear up to the age of 8–10 years, after which they tend to regress. Touraine found no cases over the age of 40, but we have seen cases over the age of 54.

(2) 'Status dysraphicus': olympian forehead, chicken chest, absence of xiphoid process, umbilical hernia, cervico-dorsal kyphosis, spina bifida, foveola coccygea, and lumbar hypertrichosis (Touraine, 1937). One or more of these anomalies were present in 47·1% of Touraine's cases and in 100% of Mercadal-Peyri's (1953) cases.

(3) Neuropsychiatric disorders: oligophrenia, emotional instability, and disturbance of character and behaviour. These were found in 90·6% of Touraine's cases and 100% of Mercadal-Peyri's cases. Cases associated with epilepsy and infantile hemiplegia have been reported. EEG anomalies have been described by Montagnani & Mariotti (1959) and also by Dociu *et al.* (1972, 1974).

Centrofacial lentiginosis is inherited as an autosomal dominant.

Differential diagnosis

In centrofacial lentiginosis, the 'butterfly-like' pattern of the pigmented spots suggests the diagnosis; as a rule (Touraine, 1941a, b, 1958), there are neither lentigines nor other pigmentary disturbances

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on the rest of the skin. Unlike ephelides the lentigines are not related to the person's natural pigmentation, are not influenced by sunlight (Siemens, 1924), and are not found all over the face, but are localized to the central face. In lentiginosis profusa (Darier), lentigines cover the entire body, and on the face they are sparse. The lentigines of the Peutz-Jeghers syndrome (lentiginosis periorificialis) are crowded in great number around the mouth and the anus, and are found also on the mucous membranes, but rarely elsewhere.

Many cases of lentiginosis are less characteristic and cannot be included in a well-defined syndrome. There are generalized varieties with local predominance: local lentiginosis of the body (involving mostly the back and shoulders); and patients with lentigines in a pseudoradicular distribution. Lentiginosis centrofacialis differs from these varieties because the lesions are not to be found on the body and the limbs.

A striking feature of all varieties of lentiginosis, centrofacial lentiginosis included, is the almost constant association with other developmental anomalies and sometimes also with neuropsychiatric disorders (Galaction-Niţelea & Dociu, 1974). Associations with congenital electrocardiographic abnormalities, mitral insufficiency, pulmonary stenosis, congenital deafness, endocrine abnormalities and skeletal malformations have been reported. For these inherited combinations of lesions, Gorlin, Anderson & Blaw (1969) proposed the name of 'Leopard syndrome'. Therefore any lentiginosis might be considered as the cutaneous signature of polydysplastic, often hereditary syndromes (Galaction-Niţelea & Dociu, 1974).

PATIENTS AND METHODS

Forty patients were studied:

Group I. Eleven patients (8 males and 3 females), aged 12–42 years, referred because of their psychiatric symptoms.

Group II. Twenty-nine patients: *subgroup A*, twenty-three teenagers (16 boys and 7 girls), aged 15–18 years, belonging to a vocational school; and *subgroup B*, six women factory workers, aged 21–36 years.

All patients were physically examined and psychologically tested. X-rays were taken of the skull and lumbosacral region. Patients in group I were examined by experts in ophthalmology, otorhino-

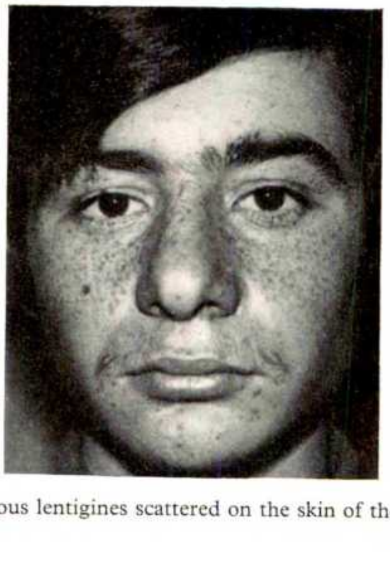

FIGURE 1. Numerous lentigines scattered on the skin of the nose and cheeks.

laryngology, and electroencephalography. The following tests were performed in some patients in both groups: urinary 17-corticosteroids, uptake by the thyroid gland of ^{131}I, scintillation scanning of the thyroid, glucose tolerance test, serum calcium and phosphate. As a control group, twenty-three boys in the same vocational school as those in group II A were similarly examined.

RESULTS

There was no difference in the degree of pigmentation or number of lentigines in patients from group I or II. All our patients had strict centrofacial localization of the lentigines with none on the rest of the skin. Four patients in group II had a 'cafe au lait' mark and two other patients had more extensive hyperpigmented patches. We could not perform any histological examinations. Dysraphism was present in 100% of group I, 92·8% of group II and 52% of the control group. The most frequent anomalies were: high palatal arch, dental malpositions, cervical or cervical-dorsal kyphosis, funnel chest, winged scapula, campto- and clinodactyly, spina bifida, hammer toes, bilateral pes cavus and a large sacral dehiscence.

X-ray examination

The twenty-three pupils with centrofacial lentiginosis were compared with the control group, and also with the other groups with centrofacial lentiginosis.

TABLE 1. Distribution of some bony abnormalities in the subjects with centrofacial lentiginosis and in the control group

	Group I	Group II		Control group of group II subgroup A
	Subjects	Total (subgroup A+B)	Subgroup A	
	11	29	23	23
	12–42 years	15–36 years	15–18 years	15–18 years
Hypomineralization tegmentum	5 (45)*	12 (41)	10 (43)	5 (21)
Digital markings on the skull	4 (36)	17 (58)	15 (65)	3 (13)
Small sella turcica	6 (54)	7 (27)	5 (21)	6 (26)
Oxycephaly	2 (18)	3 (10)	3 (13)	(0)
High vertebral bodies with a shortened anteroposterior diameter	1 (11)	5 (17)	3 (13)	(0)

* Figures in parentheses are percentages.

When calculating the percentages we took into account that in group I spinal cord radiography was performed in only nine of the eleven patients.

Neurological anomalies—group I patients only
These included: epilepsy (1 case), infantile encephalopathy with bilateral choreiform movements (1 case), dyslalia (2 cases), enuresis (3 cases).

An EEG was performed on eleven patients: it was normal in three, and showed slight slow dysrhythmia in eight cases.

Psychiatric symptoms
In group I all patients were referred because of their psychiatric symptoms. An intellectual level below the average was found in four cases, oligophrenia in three, and marked behavioural disturbances or a psychopathic condition in all the other subjects. In all group I cases there was retardation of psychomotor development (walking and speech) in early childhood. By contrast in group II A, a neurotic decompensation on a psychopathic personality background was found in only one case. In group II B, four employees had obvious neurotic troubles.

Endocrine abnormalities
Simple parenchymatous goitres confirmed by scintillation scanning were found in four patients in group I and in five patients in group II. In the control group only one goitre was diagnosed clinically. In group II B a case of mild hypothyroidism was found. The uptake of iodine yielded figures within normal values.

In ten subjects (one in group I and nine in group II) we found mild hypocalcaemia, hypocalciuria, hyperphosphataemia and hyperphosphaturia. The urinary 17-corticosteroids were reduced in two boys and three girls (1·58 mg and 5·20 mg/24 h), and were increased in four girls (12–20·7 mg/24 h).

The glucose tolerance test showed latent diabetes in two cases, and a flat curve (below 110 mg %) in two other subjects 15–17 years old. In one 36-year-old subject the blood sugar was 52 mg%; a tolbutamide test (Fajan and Conn test) induced a fall from 77 to 36 mg% at 40 min, which aroused the suspicion of an insulinoma.

In group I, we found one patient with hypophyseal dwarfism, one with bilateral ectopic testicles, and two with delayed puberty. In group II, four boys were prepubertal at the age of 16, and one had an adiposo-genital dystrophy. We also found congenital deafness in two cases (group II) and congenital ocular anomalies in nine—marked amblyopia with nystagmus, melanocytic naevus of the iris and persistence of the pupillary membrane.

Vegetative dysfunctions
Five patients in group I and seven in group II had vertigo with nausea, excessive sweating, a labile pulse and blood pressure, or damp and cyanotic extremities.

DISCUSSION

1·54% of 1500 pupils at a vocational school had centrofacial lentiginosis compared with 1·25% in Mercadal Peyri's (1953) study. There was little evidence of psychiatric disturbances in the twenty-three pupils in group II A, but they did show minor neurotic traits, similar to those in the control group. However, as far as the neuropsychiatric disturbances were concerned, this group was preselected by previous schooling, by passing of an entrance examination, and by medical selection. All the children with oligophrenia, psychopathic behaviour, or evident dysmorphism had been excluded.

Group I was also preselected because all patients were referred on account of their gross psychiatric symptoms. An intermediate situation was found in the employees aged 21–36 years, four out of six having symptoms.

Dociu *et al.* (1971) showed that patients with centrofacial lentiginosis have more bone dysmorphism of the spinal column and skull than does the rest of the population. It would seem that this is also related to the severity of psychiatric disorders. (Bone dysmorphism was more marked and frequent in group I subjects.)

In both groups endocrine disturbances were present. In 1958, Touraine described the association with adiposogenital dystrophy (Frölich's syndrome) and radiological changes in the sella turcica. Musumeci (1947) reported retarded puberty. Montagnani & Mariotti (1959) suggested that centrofacial lentiginosis was related to injury of the hypophysis. In group I we found a small sella turcica in 54% of the patients.

In both groups we found abnormal urinary 17 corticosteroids and frequently a simple goitre. Hypocalcaemia and hyperphosphaturia were often associated with vegetative dysfunctions.

The neuropsychiatric disturbances found in group II A are quite different from the classical findings in centrofacial lentiginosis. This is because Touraine, Musumeci, Montagnani and Mariotti studied patients referred with symptoms, whereas we studied subjects from school communities.

Conclusions

(1) Centrofacial lentiginosis is easy to diagnose and the patients must be investigated.

(2) There is no relationship between the number of lentigines and the severity of the psychiatric and somatic abnormalities.

(3) Dysraphism, bone developmental anomalies, and endocrine and vegetative dysfunctions are commoner in these patients.

(4) Our preselected group of twenty-three adolescents had no statistically significant psychiatric impairment compared with the control group.

REFERENCES

Dociu, I., Galaction-Niţelea, O., Şirjiţă, N. & Murgu, V. (1971) La lentiginose centro-faciale de Touraine (L.C.F.). A propos de 7 cas. *La Presse Médicale*, **79**, 1111.

Dociu, I., Galaction-Niţelea, O., Şirjiţă, N. & Murgu V. (1972) Die centrofaciale Lentiginose. Beobachtungen an 9 Patienten. *Der Hautarzt*, **22**, 389.

Dociu, I., Galaction-Niţelea, O., Şirjiţă, N. & Murgu, V. (1974) Lentiginoza centrofacială Touraine (L.C.F.) *Neurologia, Psihiatria, si neurochirurgia*, **19**, 21.

Galaction-Niţelea, O. & Dociu, I. (1974) Lentiginoses et anomalies de développement. *Dermatologica*, **141**, 108.

Gorlin, R.J., Anderson, R.C. & Blaw, M. (1969) Multiple lentigines syndrome. *American Journal of Diseases of Children*, **117**, 652.

Mercadal-Peyri (1953) quoted by Touraine (1958).

Montagnani, A. & Mariotti, F. (1959) La lentigginosi centro-facciale neuro-disrafica di Touraine (A proposito di un caso clinico). *Minerva Dermatologica*, **34**, 588.

Musumeci, V. (1947) Studio clinico e critico sulla lentigginosi centro-faciale. *Giornale Italiano di Dermatologia e Sifilologia*, **88**, 502.

Siemens, H.W. (1924) Zur Kenntnis der Epheliden mit Bemerkungen über Haarbzeichung und Haarfarbenbestimmung. *Archiv für Dermatologie und Syphilis*, **147**, 210.

Touraine, A. (1937) L'état dysraphique. *Le progrès médical*, **65**, 361.

Touraine, A. (1941a) Lentiginose centro-faciale et dysplasies associées. *Bulletin de la Société française de Dermatologie et de Syphiligraphie*, **48**, 518.

Touraine, A. (1941b) Une nourvelle neuro-ectodermose congénitale: la lentiginose centro-faciale et ses dysplasies associées. *Annales de Dermatologie et de Syphiligraphie*, **8**, 453.

Touraine, A. (1958) La lentiginose centro-facilae neuro-dysraphique. *La Presse Médicale*, **66**, 1611.

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The lipid composition of sebaceous glands as a reflection of gland size*

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SUMMARY

Following 4 h incubation *in vitro*, the patterns of incorporation of [1-^{14}C] acetate into the lipid classes of human sebaceous glands which were dissected from small skin biopsies have been established for glands of different size. It has been shown that in the larger sebaceous glands proportionately more of the labelled acetate is incorporated into squalene at the expense of triglycerides.

Experiments are presented as a result of which we conclude that this *in vitro* phenomenon, observed with [1-^{14}C] acetate incorporation, does reflect parallel changes in the proportions of these lipids actually present in glands of different size. It is suggested that the larger sebaceous glands of the acne patient elaborate sebum which has an enhanced potential for inducing comedo formation by virtue of an increased concentration of squalene.

This work also demonstrates that, in the interpretation of *in vitro* studies of sebaceous gland lipogenesis utilizing labelled precursors, the size of the sebaceous glands must be carefully considered whenever patterns of incorporation are being compared.

In 1971, a method was described for measuring the amount of [1-^{14}C] acetate incorporated into the lipids of small numbers of sebaceous glands, isolated intact by dissection from a full thickness skin biopsy following a 4 h incubation (Summerly & Woodbury, 1971). Further work (Summerly, 1974) suggested that large sebaceous glands incorporate proportionately more [1-^{14}C] acetate into squalene than small glands. In this paper, these observations have been confirmed, indicating that the largest sebaceous glands contain relatively more squalene at the expense of wax esters and triglycerides. Alternative explanations for these *in vitro* findings have been explored and eliminated.

MATERIALS AND METHODS

Incorporation of [1-^{14}C] *acetate into sebaceous gland lipids*

Skin biopsies were removed with a 3 or 5 mm diameter high speed rotary drill using local anaesthetic without adrenaline. Specimens were obtained from the upper back close to the midline in seventeen patients suffering from acne vulgaris (16 males and 1 female) and from five non-acne control volunteers (3 males and 2 females). Other specimens were taken from the scalp (1 male patient), the lower back (1 female patient) and from the nose (1 male patient with rhinophyma).

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* Based on a paper presented at the 4th course on Progress in Biological Sciences in Relation to Dermatology, Cambridge, September 1973.

After incubation with [1-^{14}C] acetate and treatment with calcium chloride solution, the sebaceous glands were dissected out and the uptake of acetate into the various lipid classes was measured as described previously (Summerly & Woodbury, 1971; Summerly, 1974).

Measurement of sebaceous gland size

A Griffin stereomicroscope fitted with an eyepiece micrometer was used, the 'stage' dimension of the side of each micrometer square being 0·5 mm. After the sebaceous glands in each biopsy had been dissected free from the overlying epidermis and the deeply lying glands recovered from the dermis, the individual glands were arranged over the dissecting stage in order that the size of a gland could be compared with the area of a square. Glands covering less than half a square were graded as *small*, *medium* glands covered between half and the whole of a square, *large* glands covered between one and two squares and *massive* glands covered two or more squares.

After measurement under the microscope, each sebaceous gland was transferred to a small square of aluminium foil and weighed using a model M-10 Cahn Electrobalance. Good correlation was observed between the assessment of gland size using the eyepiece micrometer and the weight of the gland. Small glands weighed less than 0·03 mg, medium glands weighed 0·03–0·06 mg, large glands weighed 0·08–0·11 mg, and massive glands weighed 0·17–0·29 mg. After the sebaceous glands from a biopsy had been graded in these two ways, they were transferred to separate tubes so that each tube contained glands of one size range.

Lipid analysis

Lipids were extracted (Folch, Lees & Sloane-Stanley, 1957; Ansell & Hawthorne, 1964) and separated chromatographically as previously described (Summerly & Woodbury, 1971; Summerly, 1974) on thin layer plates that had been cleared of extraneous lipid by developing the plates twice in the same solvent mixture that was subsequently used in the separation procedure. Labelled extracts and reference compounds were run alongside. After chromatography and autoradiography, the silica gel was scraped from those areas on the plate which corresponded with triglycerides, wax esters and squalene. The lipids were then recovered from the silica gel by extracting with three 5 ml portions of chloroform: methanol (2:1 by volume). Kritchevsky & Malhotra (1970) have reported 90–100% recovery of neutral lipids from silica gel using this method. Each extract was evaporated to dryness at 37°C under a stream of oxygen-free nitrogen and then redissolved in a small volume of chloroform: methanol and the solution transferred to small squares (1·5 × 1·5 cm) of previously weighed absorbent paper (Brittains Ltd., Leek, Staffordshire). This procedure was then twice repeated to ensure the transfer of all the lipid onto the absorbent paper. The paper was then weighed again on a Cahn model M-10 electrobalance and the weight of the lipid obtained by difference.

RESULTS

Excretory and membrane lipids

The lipids synthesized by sebaceous glands can be divided into two groups. One group provides structural elements of cell membranes and includes the polar lipids, free sterols and free fatty acids. The other group is exported in sebum and comprises the triglycerides, sterol and wax esters and squalene. In this study, we sought to detect minor differences in the relative proportions of the excretory lipids, differences which could conceivably have been masked by alterations in the membrane lipid contribution. For example, we have demonstrated that in the small sebaceous glands a significantly greater proportion of the total [1-^{14}C] acetate incorporated appears in the membrane lipids (a mean value of 17·4%); as compared to a mean value of 11·9% in the large glands (unpublished observations). Consequently, we have expressed the incorporation of precursor into a particular excretory lipid class as a percentage of the incorporation into all the excretory lipids. Likewise, the results of lipid analysis are expressed as a percentage of the total excretory lipid present. In the results section, all the wax ester values include a small contribution of sterol esters, which cannot be resolved from the wax esters in the chromatographic separations we have used.

Sebaceous gland size and the pattern of incorporation of [1-^{14}C] *acetate into excretory lipids*

The results are presented in Fig. 1 and Table 1. In most biopsies sebaceous glands of two sizes were recovered, the commonest combination being 'small', dissected from the epidermal disc attached to minute sebaceous follicles or to a terminal hair follicle, and 'large', recovered from the dermal component. The percentage incorporation of [1-^{14}C] acetate during 4 h into squalene was significantly greater in massive glands ($P<0\cdot001$) and large glands ($P<0\cdot05$) compared with small glands. There was a concomitant decrease in the percentage incorporation of [1-^{14}C] acetate into triglycerides in massive glands ($P<0\cdot005$), large glands ($P<0\cdot001$) and medium glands ($P<0\cdot05$) compared with small glands.

The validity of results from 4 h incubation experiments

It may be objected that incorporations of [1-^{14}C] acetate into lipids during a 4 h incubation period do not reflect the total amounts of lipid present in a sebaceous gland. This objection has been met by comparing 4 h with 24 h incubation results, and also by comparing 4 h incubation results with lipid analyses.

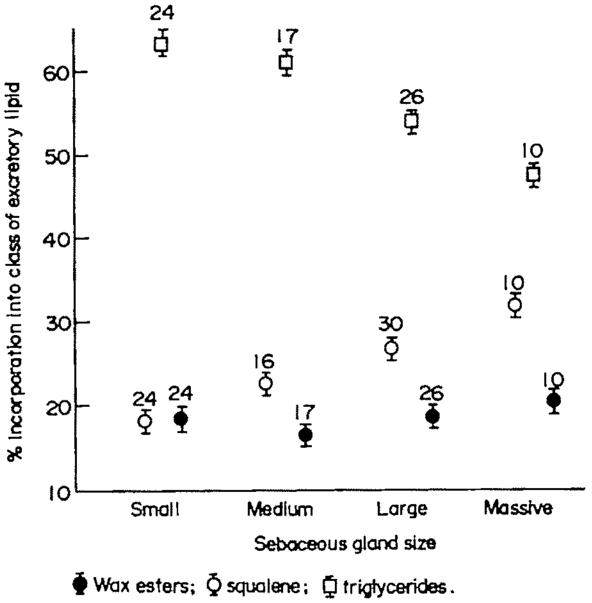

FIGURE 1. Graph showing the percentage of the [1-^{14}C] acetate incorporated into the three classes of excretory lipid in each size of sebaceous gland. Results are expressed as mean ± s.e. mean with the number of observations stated.

TABLE 1. Effect of gland size on the incorporation of [1-^{14}C] acetate during a 4 h incubation into the excretory lipids of sebaceous glands

	Incorporation as % of total uptake into excretory lipids		
Gland size	Triglyceride	Wax ester	Squalene
Small (24)	64·0±1·4	18·1±1·3	17·9±0·8
Medium (17)	60·9±1·9	16·7±1·2	22·4±1·4
Large (26)	55·0±1·4	18·5±1·1	26·5±1·1
Massive (10)	47·8±1·6	20·4±0·9	31·8±1·5

Results expressed as mean ± s.e. mean with the number of observations in parentheses.

In nine experiments, two specimens were placed in each incubation bottle. After 4 h, one specimen was removed and processed; the other specimen was incubated for a further 20 h before being processed. All the sebaceous glands from each biopsy were processed together and it was carefully noted at dissection that the range of gland sizes from each biopsy was similar. The results, shown in Table 2, show that the pattern of incorporation after a 24 h incubation does not differ significantly from the 4 h pattern.

TABLE 2. Effect of time on the pattern of incorporation of [1-^{14}C] acetate into the excretory lipids of sebaceous glands

Duration of Incubation (h)	Incorporation as % of total uptake into excretory lipids		
	Triglyceride	Wax ester	Squalene
4 (9)	64·9±2·0	15·8±0·9	19·3±2·2
24 (9)	66·3±1·3	16·0±0·7	17·7±1·2

Results expressed as mean ± s.e. mean with the number of observations in parentheses.

In a second series of experiments, narrow lengths of midline dorso-lumbar skin removed at laminectomy and full thickness slices of nasal skin and rhinophyma tissue were used. From each specimen, two 5 × 5 mm squares of skin were incubated with [1-^{14}C] acetate for 4 h and were subsequently dissected and processed. The predominant gland size of the yield was recorded. The remainder of each specimen was then also cut into small squares and these were floated on 1 mol/litre calcium chloride for 2 h and as many intact sebaceous glands as possible harvested. The predominant gland size was recorded and the wet weight of the gland yield noted: specimens of lumbar skin yielded 1·1–2·4 mg of sebaceous glands while the rhinophyma tissue yielded 203 mg. The sebaceous glands from each specimen were then analysed for triglyceride, wax ester and squalene content as described in the methods section. Table 3 compares the incorporation of [1-^{14}C] acetate into the excretory lipids with the amounts of these lipids actually present. It can be seen that there is reasonable agreement between the two sets of values.

By having noted the predominant gland size in the yields of sebaceous glands obtained from the small squares of skin selected for incubation with [1-^{14}C] acetate and in the bulked gland harvest

TABLE 3. Incorporation of [1-^{14}C] acetate during a 4 h incubation into the excretory lipids of sebaceous glands, and the distribution by weight of these lipids in the glands

	Triglyceride	Wax ester	Squalene
Incorporation of [1-^{14}C] acetate into excretory lipids of sebaceous glands as a % of total uptake into excretory lipids (5)	51·1 ± 3·9	22·8 ± 1·0	25·7 ± 3·6
Lipid composition by weight of isolated sebaceous glands as a % of the total weight of excretory lipids (6)	42·7 ± 2·2	25·8 ± 1·3	31·5 ± 2·3

Results expressed as mean ± s.e. mean with the number of observations in parentheses.

from the remainder of the skin specimen it has been possible to compile Table 4. It can be seen that the relationship between gland size and the percentage incorporation into squalene, previously demonstrated in Table 1, still obtains and that a similar trend is evident in the actual amounts of squalene present in the yields, in which the predominant gland size can be seen to vary.

Absence of an effect due to the geometry of the incubation conditions on the uptake of [1-^{14}C] *acetate into the excretory lipids of sebaceous glands of different sizes*

In all our incubations, the skin was placed dermis side down on a perforated stainless steel bridge, so that only the lowest 1–2 mm of dermis was in contact with the incubation medium. As the largest sebaceous glands are invariably located in the deeper parts of the dermis, it is possible that these glands were more favourably placed to incorporate labelled acetate than the small superficially located glands.

To test this possibility, two biopsy specimens were incubated in the same bottle. One specimen was placed in the usual orientation and the other was placed upside down on the stainless steel bridge. The patterns of incorporation obtained from the large sebaceous glands located in the deep dermis of each

TABLE 4. Relationship between predominant gland size of sebaceous gland yield and squalene proportion

Predominant gland size	Squalene proportion expressed as % of total uptake of [1-^{14}C]acetate into excretory lipids	Squalene proportion expressed as % of total weight of excretory lipids
Small and medium	—	27·7
Medium	22·8, 16·8	22·7, 29·0
Medium and large	24·3	35·4, 38·8
Massive	38·5, 27·3	35·2

specimen were identical, as were the patterns obtained from the appendage-freed specimens of epidermis.

Pattern of incorporation of D-glucose-U-[^{14}C] *into the excretory lipids of sebaceous glands*

In a single experiment, eight 5 × 5 mm squares of full thickness skin trimmed of subcutaneous fat were selected from a length of skin removed from the edge of a midline incision in the lumbo-dorsal region prior to laminectomy. Four specimens were incubated for 4 h in the presence of 29 μCi [1-^{14}C] acetate and four in the presence of 25 μCi D-glucose-U-[^{14}C] and then dissected and processed as previously described. The glands were small to medium in size. All the sebaceous glands harvested from one skin specimen were processed together. The patterns of incorporation into the excretory lipids are shown in Table 5. The percentage incorporation into wax esters from D-glucose-U-[^{14}C] was significantly reduced ($P<0{\cdot}05$) compared with the incorporation from acetate.

TABLE 5. Incorporation of [1-^{14}C] acetate and D-glucose-U-[^{14}C] into the excretory lipids of sebaceous glands during a 4 h incubation

	Incorporation as % of total uptake into excretory lipids		
Precursor	Triglyceride	Wax ester	Squalene
[1-^{14}C]acetate (4)	63·0 ± 2·3	22·2 ± 1·1	14·8 ± 2·2
D-glucose-U-[^{14}C] (4)	72·6 ± 3·6	12·9 ± 2·2	14·5 ± 1·8

Results expressed as mean ± s.e. mean with the number of observations in parentheses.

DISCUSSION

The results obtained and the conclusions drawn from a study of the lipid synthesizing capacity of skin components *in vitro*, separated by dissection procedures after short-term maintenance of whole skin, are perforce artificial. Earlier workers (Griesemer & Thomas, 1966) conceded that the performance of skin *in vitro* represented baseline metabolism which possibly operated only in the *in vitro* situation or when the skin was at rest physiologically. They, nevertheless, concluded that short-term incubation *in vitro* provided a reliable marker of lipogenesis and was suitable for observing the effects of various factors. Much of our current knowledge of cutaneous lipogenesis (pathways employed, substrates utilized, essential co-factors, sites of elaboration of particular lipids) has been derived from the *in vitro* techniques (Wheatley, 1974). In at least one other aspect of metabolism at cellular level, studies of DNA synthesis in which the incorporation of tritiated thymidine is widely used, workers have shown a close correlation between the results obtained *in vitro* and *in vivo* (Lachapelle & Gillman, 1969; Hell & Maibach, 1972). In our continuing studies of lipogenesis in the dissected sebaceous gland, utilizing [1-^{14}C] acetate incorporation *in vitro* to monitor this metabolism, we have attempted to explore fully the significance of any pattern of incorporation obtained. In earlier work the highly contrasting patterns obtained from the dissected sebaceous glands, appendage-freed epidermis, sebocysts and wens have permitted logical and functional interpretations (Summerly & Woodbury, 1972; Summerly, 1974).

In the present study we have demonstrated that in the sebaceous glands during short-term maintenance *in vitro*, of the labelled acetate incorporated into the excretory lipids, the percentage which appears in squalene increases with increasing gland size at the expense of triglycerides, the wax ester proportion remaining unchanged. The other experiments described were designed to elucidate further the significance of this finding.

The possibility that, in the different sizes of sebaceous gland, the results obtained simply reflected changes in the proportion of sebaceous cells in the centre of a lobule and undifferentiated cells at the periphery can be excluded; our results, as expressed in this study, indicate only alterations within the excretory lipids. A diffusion artefact due to the proximity of the largest sebaceous glands to the maintenance medium has been excluded by the constancy of the pattern obtained in the different sizes of sebaceous gland and epidermis irrespective of the location of these components in relation to the medium.

Following several days starvation in obese subjects (Pochi, Downing & Strauss, 1970) and in normal volunteers (Downing, Strauss & Pochi, 1972) selective inhibition of sebaceous lipogenesis resulted in a dramatic rise in the proportion of squalene in the skin surface lipid and a corresponding fall in triglyceride. Hsia, Drieze & Marquez (1966) have reported that the incorporation of [1-^{14}C] acetate *in vitro* into the lipids of excised skin specimens was impaired after only 18 h of fasting prior to biopsy. Downing & Strauss (1974) postulate that squalened concentration, the most variable aspect of sebum composition, might be affected by normal overnight fasting, a change which would not be detected because of the extended period during which mixing in the pilosebaceous canal could occur. All the drill biopsy specimens used in this study were taken in the mornings 2–3 h after breakfast whereas in those operation specimens, in which low squalene percentages have been encountered (Table 5), overnight fasting was the rule. It was conceivable that the largest sebaceous glands could have responded much earlier to the lack of essential dietary precursors *in vitro*, even after 4 h maintenance, resulting in a decrease in the synthesis of triglycerides with a corresponding increase in the percentage incorporation into squalene. This explanation for our results can perhaps be excluded by our failure to detect increased levels of incorporation into squalene after 24 h maintenance (Table 2).

The constancy of the distribution of the incorporated [1-^{14}C] acetate in the excretory lipids during continuing lipogenesis *in vitro* which we have demonstrated after 4 h, 24 h (Table 2) and, in 3 instances, 52 h (Summerly, 1974) suggests that this pattern of incorporation would correlate with the relative amounts of these lipids actually present in the sebaceous cells. In cutaneous lipogenesis *in vitro*, the precursor [1-^{14}C] acetate may form acetyl-CoA directly or after mitochondrial citrate synthesis, or it may enter the Krebs cycle where C-2 is lost as carbon dioxide and C-1 remains as the carboxyl group on the oxaloacetate; whereas D-glucose-U-[^{14}C] may generate pyruvate, $NADPH_2$, $NADH_2$ and α-glycerophosphate (Wheatley, 1974). Table 5 shows that the patterns of incorporation produced by these two labelled precursors, whose entry points in the pathways of lipid synthesis are known to be different, were similar with respect to squalene and triglycerides, the lipid classes in which we were especially interested; thus lending some support to the claims that the patterns we have previously obtained with [1-^{14}C] acetate do reflect the relative amounts of excretory lipids present.

From the evidence already discussed we infer that the sebaceous cells in the largest sebaceous glands synthesize excretory lipid containing a greater proportion of squalene, which would be reflected in the sebum composition, a change which in the acne subject is associated with a greater potential for comedone production. Absolute proof of an alteration in the excretory lipid profile with increasing sebaceous gland size would require a biochemical analysis of the lipids of individual sebaceous glands which would be a formidable undertaking if one considers that the weight of a single small sebaceous gland, as opposed to its lipid yield, was less than 0·03 mg and the largest weight recorded for a single 'massive' gland in this series was 0·29 mg. Nevertheless if a close correlation could be demonstrated between the relative amounts of excretory lipids present in a bulk extract of sebaceous gland lipids and the pattern of incorporation of [1-^{14}C] acetate obtained from this same yield; and if an increasing proportion of one particular lipid in the biochemical analysis was similarly reflected in the *in vitro* studies, our premise that the pattern of incorporation of [1-^{14}C] acetate monitored the relative amounts of these lipids in the gland would be proven. Such evidence is presented in Tables 3 and 4.

Planimetry studies have demonstrated that the cross-sectional areas of the largest sebaceous glands on the backs of acne patients are greater than in non-acne subjects (Plewig, 1974). We therefore suggest, from the present study, performed in the main by utilizing skin from the upper back of male acne patients, that the largest of these sebaceous glands, which on dissection are often seen to be associated with comedone formation, synthesize an excretory product containing an increased proportion of squalene. Our findings based on *in vitro* studies of [1-^{14}C] incorporation and lipid analysis correlate well with the finding of an increased percentage of squalene in the forehead skin surface lipid of acne patients by two groups of workers (Cotterill *et al.*, 1972; Gloor *et al.*, 1972). An increase in the concentration of any one of a number of the constituents of sebum may, as inferred by Kligman, Wheatley & Mills (1970) using the rabbit ear canal model, bestow enhanced comedogenic potential to the sebum as a whole. Under these test conditions in which 25% concentrations were applied to the rabbit ear, squalene was observed to have comedogencity second only to free fatty acids.

A highly important practical point emerges from this study. If the effects of altering the physical and chemical milieu of the sebaceous glands or the actions of drugs are studied using a technique which involves a comparison between the performances *in vitro* of small numbers of sebaceous glands, it is essential that due consideration is given to the size of the glands when results are being interpreted.

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REFERENCES

ANSELL, G.B. & HAWTHORNE, J.N. (1964) *Analytical Methods, in the Phospholipids*, p. 42. Elsevier Publishing Co., Amsterdam.

COTTERILL, J.A., CUNLIFFE, W.J., WILLIAMSON, B. & BULUSU, L. (1972) Further observations on the pathogenesis of acne. *British Medical Journal*, **iii**, 444.

DOWNING, D.T. & STRAUSS, J.S. (1974) Synthesis and composition of surface lipids of human skin. *Journal of Investigative Dermatology*, **62**, 228.

DOWNING, D.T., STRAUSS, J.S. & POCHI, P.E. (1972) Changes in skin surface lipid composition induced by severe caloric restriction in man. *American Journal of Clinical Nutrition*, **25**, 365.

FOLCH, J., LEES, M. & SLOANE-STANLEY, G.H. (1957) A simple method for the determination and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, **226**, 497.

GLOOR, M., GRAUMANN, U., KIONKE, M., WIEGAND, I. & FRIEDERICH, H.C. (1972) Skin surface lipids: qualitative and quantitative determination in patients with acne vulgaris and controls. I. *Archiv für Dermatologische Forschung*, **242**, 316.

GRIESEMER, R.D. & THOMAS, R.W. (1966) Lipogenesis in human skin. IV. *in vitro* rate studies. *Journal of Investigative Dermatology*, **47**, 432.

HELL, E. & MAIBACH, H. (1972) A comparison of *in vivo* and *in vitro* methods of identifying human epidermal cells in DNA synthesis. *British Journal of Dermatology*, **86**, 506.

HSIA, S.L., DRIEZE, M.A. & MARQUEZ, M.C. (1966) Lipid metabolism in human skin. II. A study of lipogenesis in skin of diabetic patients. *Journal of Investigative Dermatology*, **47**, 443.

KLIGMAN, A.M., WHEATLEY, V.R. & MILLS, O.H. (1970) Comedogenicity of human sebum. *Archives of Dermatology*, **102**, 267.

KRITCHEVSKY, D. & MALHOTRA, S. (1970) Recovery of lipids from thin-layer chromatography for radioassay. *Journal of Chromatography*, **52**, 498.

LACHAPELLE, J.M. & GILLMAN, T. (1969) Tritiated thymidine labelling of normal human epidermal cell nuclei: a comparison, in the same subjects of the *in vivo* and *in vitro* techniques. *British Journal of Dermatology*, **81**, 603.

PLEWIG, G. (1974) Acne vulgaris: proliferative cells in sebaceous glands. *British Journal of Dermatology*, **90**, 623.

POCHI, P.E., DOWNING, D.T. & STRAUSS, J.S. (1970) Sebaceous gland response in man to prolonged total caloric deprivation. *Journal of Investigative Dermatology*, **55**, 303.

SUMMERLY, R. (1974) Skin components: ^{14}C acetate incorporation *in vitro*, with particular reference to lipid synthesis in the isolated (dissected) human sebaceous gland. *Transactions of the St John's Hospital Dermatological Society*, **60**, 1.

SUMMERLY, R. & WOODBURY, S. (1971) The *in vitro* incorporation of ^{14}C acetate into the isolated sebaceous glands and appendage-freed epidermis of human skin: a technique for the study of lipid-synthesis in the isolated sebaceous gland. *British Journal of Dermatology*, **85**, 424.

SUMMERLY, R. & WOODBURY, S. (1972) Lipid synthesis (^{14}C-acetate incorporation) in the isolated human sebaceous gland, the appendage-freed epidermis, the sebocyst (steatocystoma) and the wen (keratinous cyst of skin). *British Journal of Dermatology*, **86**, 614.

WHEATLEY, V.R. (1974) Cutaneous lipogensis. Major pathways of carbon flow and possible inter-relationships between the epidermis and sebaceous glands. *Journal of Investigative Dermatology*, **62**, 245.

British Journal of Dermatology (1976) **94**, 55.

Specific cell-mediated immunity in the laboratory diagnosis of dermatophytic infections

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SUMMARY

The leukocyte adherence inhibition test was used to detect cell-mediated immunity against antigens of the infecting fungi in patients with dermatophytosis. The species-specific diagnosis thus obtained was in all cases consistent with the eventual results of mycological examination. Most of the patients also produced a specific circulating blocking factor, the detection of which may be a useful guide to the success of treatment. Immunological reactivity against *Candida albicans* was also observed.

Specific diagnosis of dermatophytosis usually depends on the cultivation and identification of the infecting fungus. Conventional tests for immunity in the patient are not often performed, since they give no reliable indication of present or past infection. Thus the examination of serum for anti-fungal antibodies is not a useful diagnostic procedure and skin tests for immediate or delayed hypersensitivity are of limited value; false negative reactions are frequently encountered with the latter type of test, notably in chronic infections (Longbottom, Murray & Pepys, 1968).

In vitro tests for cell-mediated immunity (CMI) offer a possible alternative but available methods suffer from technical difficulties, especially the delay of up to several days before results are obtained. The leukocyte adherence inhibition (LAI) test, devised originally for the detection of CMI against tumour antigens (Halliday & Miller, 1972; Halliday, Maluish & Isbister, 1974), has recently been applied to the study of immune responses to dermatophytes in man (Walters, Chick & Halliday, 1974). Further work with a new and larger series of patients indicates that the reactions are species-specific and that the nature of the infecting fungus can be reliably determined in a few hours. Moreover, a distinction between acute and chronic infections can be made on the basis of the behaviour of the patient's serum in the LAI test, and this knowledge may lead to more rational treatment.

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TABLE I. Summary of patients' histories

Patient no.	Age	Clinical presentation	Duration of complaint (years)	Treatment of patients with fungal infection	Response to treatment
1	53	Nummular dermatitis of limbs and trunk	30	Not applicable*	—
2	60	Pruritis ani	30	Not applicable*	—
3	57	Erythemato-squamous rash under both feet and between toes. Had *T. mentagrophytes* infection in 1967, treated with griseofulvin	20	Not treated by dermatologist	—
4	53	Diabetic; perianal and inguinal intertrigo	'Many'	Not applicable*	—
5	25	Vesicular rash on dorsum of big toe; maceration between toes	4	Local applications	Adequate control
6	65	Erythematous rash on upper inner thighs	20	Not treated by dermatologist	—
7	56	Scaling between toes	30	Not applicable*	—
8	61	Psoriatic inguinal intertrigo. *E. floccosum* infection on feet 3 years previously	4–5	Local applications	Adequate control
9	51	Perianal and inguinal intertrigo; psoriasis	10	Local applications	Adequate control
10	59	Scaling under foot	6	Griseofulvin 0·75 g/day for 3 months	Cured
11	54	Scaling on toes; maceration between toes. Treated 12 months previously with griseofulvin	6	Local treatment advised	Adequate control
12	48	Scaling under feet; maceration between toes	29	Not treated by dermatologist	—
13	49	Erythemato-squamous macular rash on upper inner thighs and feet	30	Griseofulvin 0·5 g/day for 6 months	Cured
14	55	Erythemato-squamous rash on palms and soles; dystrophic nails	30	Griseofulvin 0·5 g/day (July 1971); 0·75 g/day (January 1972); 1·0 g/day	Some nails cured; continued scaling of palms and soles

Table 1 *continued*

Patient no.	Age	Clinical presentation	Duration of complaint (years)	Treatment of patients with fungal infection	Response to treatment
				(April 1972); 1·125 g/day (October 1973)	(June, 1974)
15	42	Maceration and scaling between toes	22	Not treated by dermatologist	—
16	48	Vesicular dermatitis of the feet	30	Griseofulvin 0·5 g/day for 2 months	Controlled
17	52	Erythemato-squamous rash on face[a] and upper inner thighs.[b]	(a) 23 (b) 30	Griseofulvin 0·5 g/day (July 1973); 1·0 g/day (December 1973)	(a) Cured (b) Present but less marked (September 1974)

* Antifungal treatment not given since no fungi were detected (see Table 2).

PATIENTS, MATERIALS AND METHODS

Seventeen patients (16 male, 1 female) with a variety of dermatological conditions (Table 1) were examined. The men were ex-servicemen and the woman (No. 10) was a war widow. Most of the patients dated their complaint from the time of their war service but mycological investigations were not done until several years had elapsed after their discharge from the service. Patients were referred to the dermatologist either for treatment or for diagnosis.

Local applications and prolonged griseofulvin therapy partially controlled the condition in two men (Nos 14 and 17). Neither patient was taking drugs known to reduce the efficacy of griseofulvin. Patient No. 14 had taken griseofulvin for 3 years (up to 1·125 g/day) and when seen in July 1974, still had infected nails, palms and soles.

Mycological examination

Standard laboratory methods were used to detect dermatophytic fungi and *Candida albicans* (Conant *et al.*, 1971; Hazen, Gordon & Reid, 1970; Dunne, 1962). Skin scrapings were examined microscopically in 10% potassium hydroxide solution, and cultures were made in Sabouraud's glucose agar with and without antibiotics. The average time taken for isolation and identification of dermatophytes was 14 days.

Collection of leukocytes and serum

Blood was obtained by venepuncture in 20 ml quantities and mixed gently with 0·2 ml of heparin (Anticlot, Clinton Laboratories, Los Angeles, Calif.) and 0·5 ml of dextran DR1 (Macrodex, 6% in 5% dextrose, Pharmacia, Sweden). After sedimentation at 37°C for 1 h in an inverted plastic syringe, the leukocyte-rich plasma was expressed through a bent needle into centrifuge tubes. The plasma was centrifuged at about 200 *g* for 10 min. The packed cells were dispersed briefly in 1 ml of distilled water to lyse erythrocytes and 10 ml of Medium 199 (Commonwealth Serum Laboratories, Melbourne), containing 10% fetal calf serum and 0·28 mg/ml sodium bicarbonate, were quickly added. This medium was used throughout the procedure. The cells were centrifuged again and resuspended in medium to a density of approximately 2×10^7/ml. Serum was obtained from clotted blood; it was not heat-treated.

Antigen solutions

Stock antigen solutions were prepared by grinding 7·5 mg portions of powdered allergens (Hollister-Stier Laboratories, Spokane, Wash.) with a few drops of medium, then diluting to 100 ml. The final concentration of antigens in the test was 25 μg/ml. Antigens from *Trichophyton rubrum*, *T. mentagrophytes*, *Epidermophyton floccosum* and *C. albicans* were used, corresponding to the fungi most commonly encountered in this laboratory.

The LAI test

The test is slightly modified from the original method for human leukocytes (Halliday *et al.*, 1974). Equal volumes (0·1 ml) of leukocyte suspension (2×10^6 cells), antigen solution and serum were mixed in small plastic tubes and incubated at 37°C for 1 h with intermittent shaking. The serum was either normal human serum or the patient's own serum. After the preliminary incubation, the mixtures were introduced into the counting chambers of haemacytometers (W. Schreck, Hofheim, Germany). A separate haemacytometer was used for each mixture and when filled was incubated at 37°C for 1–2 h, in a large Petri dish with damp filter paper to prevent drying.

The nucleated cells were counted microscopically at a magnification of ×400 in a predetermined pattern of 16 squares, each 0·2×0·2 mm. The haemacytometer slides were then rinsed, after removal of the coverslip by flotation. This was done by slowly immersing the haemacytometer, held horizontally, into a dish containing medium or balanced salt solution. The liquid flowed under the coverslip, which floated away. The slide without coverslip was then held by one end and slowly immersed into a beaker of medium, withdrawn, reversed and again immersed and withdrawn. After a drop of medium was put on the slide, a clean coverslip was put in place. It is convenient to use 24 mm square No. 1 coverslips instead of the heavier haemacytometer type.

The adherent cells remaining on the slide were counted in the *same* squares as examined previously. The percentage of adherent cells for each square, the mean, and the standard error (s.e.) of the mean were calculated.

All counting was done with coded samples, before the eventual mycological diagnosis was known. A reduced count (inhibition of adherence) indicates reactive leukocytes or positive CMI; restoration of the count towards the normal control, in the presence of patient's serum, indicates a blocking factor in the serum.

RESULTS

Table 2 summarizes the results obtained with nine of the patients studied. LAI tests were performed with four different antigens and a control with no added antigen. The mycological diagnosis is also reported. In every case where a dermatophyte was isolated and identified, the patient had previously given a positive specific LAI test, i.e. the degree of adherence was reduced with the corresponding fungal antigen. Where no dermatophytes were isolated, no reactivity was found with dermatophyte antigens. The cultivation of *C. albicans* was associated with a positive LAI test with this antigen in one case (No. 9), but not in two others (Nos 6 and 8). Reactivity with *C. albicans* antigen was evident on other occasions, such as with patients 3, 4, 5 and 7, when the organism was not detectable by cultural examination.

TABLE 2. Reactivity of patients' leukocytes in LAI tests

Patient no.	Mean % adherence (±s.e.) with fungal antigens*					Culture
	Control	*T. rubrum*	*T. mentagrophytes*	*E. floccosum*	*C. albicans*	
1	78·1±3·0	76·5±2·6	78·8±2·3	82·2±1·6	79·0±2·6	Negative
2	78·2±2·7	78·4±2·5	85·7±1·9	78·9±1·8	75·6±4·8	Negative
3	59·8±3·9	*32·6±3·3*	51·7±2·3	66·6±3·2	*46·6±2·6*	*T. rubrum*
4	59·9±1·1	53·6±1·5	68·9±2·1	70·9±2·1	*42·1±1·1*	Negative
5	74·2±1·3	70·2±2·3	*31·5±1·8*	70·3±1·4	34·5±1·1	*T. mentagrophytes*
6	76·8±2·8	66·3±4·1	62·7±3·5	70·2±2·7	63·9±2·7	*C. albicans*
7	73·6±1·8	76·6±1·5	72·5±2·7	72·3±1·9	27·0±1·6	Negative
8	68·1±2·3	69·6±2·0	66·5±2·9	*24·5±1·5*	59·6±3·7	*C. albicans*†
9	58·1±2·1	59·9±2·0	52·3±3·2	62·7±3·2	33·1±2·0	*C. albicans*

* Positive reactions (significantly reduced adherence) are shown in italics.

† *E. floccosum* had been isolated 3 years previously.

TABLE 3. Reactivity of leukocytes in LAI tests with and without patient's own serum

Patient no.	Mean % adherence (±s.e.) with fungal antigens*					Culture
	Control	*T. rubrum*	*T. mentagrophytes*	*E. floccosum*	*C. albicans*	
10	69·0±1·9 (76·7±2·9)	48·4±2·9 (78·8±1·5)	60·6±2·4 (70·6±3·1)	68·7±2·0 (72·5±2·6)	45·5±2·4 (62·4±2·7)	*T. rubrum*
11	73·7±3·1 (69·9±2·0)	79·0±2·9 (73·5±2·8)	79·1±4·1 (74·1±2·6)	38·4±2·8 (52·1±5·8)	39·0±3·3 (32·3±1·3)	*E. floccosum*
12	70·4±1·5 (73·9±2·2)	63·2±1·6 (77·3±1·9)	65·8±1·9 (81·1±1·4)	45·9±1·5 (81·3±1·3)	49·1±1·4 (43·7±1·7)	*E. floccosum*
13	70·8±8·3 (76·9±1·6)	29·5±1·4 (61·0±2·3)	76·7±2·0 (71·5±1·8)	72·6±1·5 (Not done)	32·4±2·0 (25·9±1·8)	*T. rubrum*
14	66·5±2·2 (75·2±2·5)	32·0±2·6 (74·8±1·5)	75·8±2·3 (67·1±2·6)	69·7±2·6 (67·4±1·9)	46·7±3·6 (37·6±1·5)	*T. rubrum*
15	75·7±1·4 (74·2±1·8)	74·2±1·4 (73·7±1·1)	41·3±2·4 (57·4±2·5)	71·8±1·3 (73·4±1·2)	56·3±1·3 (53·0±1·2)	*T. mentagrophytes*
16	65·3±1·3 (70·3±2·9)	75·6±2·1 (72·4±2·6)	37·6±2·2 (46·8±1·7)	69·5±1·6 (70·6±2·6)	48·6±2·3 (61·1±2·9)	*T. mentagrophytes*
17	56·4±3·4 (80·2±2·3)	39·1±3·1 (73·9±2·4)	76·5±2·0 (67·0±2·9)	70·0±2·5 (66·5±2·7)	64·7±2·3 (65·3±2·2)	*T. rubrum*

* Positive reactions (significantly reduced adherence with antigen and significantly restored with serum are shown in italics. Data obtained with the patient's serum added are in parentheses.

A further series of eight patients was studied in the same way, but the LAI test was extended to include each patient's own serum in the leukocyte–antigen mixtures. The results are shown in Table 3. Again the immunological test predicted exactly the dermatophyte later isolated. *C. albicans* was not isolated from these patients, but almost all of them had leukocytes reactive with this antigen. When autochthonous serum was included, complete or partial blocking of the LAI reaction was found in the dermatophyte reactivity of all patients except No. 16, where the effect was marginal. Serum blocking of reactivity to *C. albicans* was evident in patients 10 and 16 but not in the others.

DISCUSSION

This paper describes the application of an *in vitro* test for CMI to the immunodiagnosis of mycotic infection. The LAI test is a simple and rapid procedure, which has predicted with excellent consistency the outcome of the more tedious mycological examinations for dermatophytic fungi.

The reliability of the technique described depends on a number of considerations. First, the specificity of the antigens used will determine whether or not infections caused by different species of dermatophytes can be distinguished. In our experience, the crude preparations available commercially appeared to contain suitable species-specific antigens. Patients with infections caused by one species, confirmed by mycological examination, reacted in LAI tests with the antigen preparation from this species only (Tables 2 and 3). Further tests with a greater range of antigens should be done to extend this observation. The possibility of double infections must also be borne in mind, although no evidence for these was apparent in the patients examined. Species-specificity of immunological reactions in dermatophytosis is a most unusual finding, these fungi generally exhibiting cross-reacting antigens in conventional serological tests (Grappel, Bishop & Blank, 1974).

The second consideration is that the test should be positive in all stages of infection and negative in normal individuals. We have found an excellent correlation between unequivocal cellular reactivity in LAI and clinical and mycological evidence of current infection, in both acute and chronic conditions. More information is desirable on 'normal' individuals, since it is likely that previous or inapparent infection would induce an immune response and lead to a positive reaction. Among the patients examined, six showed no reactivity to the dermatophyte antigens at the time when no dermatophytic fungus could be cultured (Table 2) but No. 8 was reactive to *E. floccosum*, apparently as a result of known previous infection. In this case, it is possible that overgrowth of culture plates with *C. albicans* may have prevented recognition of the slow-growing dermatophyte. Patient No. 3 had been infected previously with *T. mentagrophytes* but showed no clear immunological evidence of this.

As described previously (Walters *et al.*, 1974) and elaborated in this paper, chronic dermatophytosis led to the appearance in the serum, of a blocking factor which abrogated the LAI reaction *in vitro*. Our earlier studies demonstrated the specificity of blocking. In tests performed without the patient's own serum, CMI was still readily detectable. The *in vivo* presence of blocking—rather than the lack of CMI—probably explains the poor response to skin tests for delayed hypersensitivity, as usually found in chronic cases (Jones, Reinhardt & Rinaldi, 1973). A quite different phenomenon was observed here in LAI tests using Candida antigen; although cellular reactivity was commonly observed even when this fungus was not isolated (possibly as a result of past infection or reaction to commensal yeast flora), confirmed infection with *C. albicans* was associated with specific non-reactivity or anergy of the leukocytes in two patients. Blocking of *C. albicans* CMI was found with serum from two others. Because of these complications, the LAI test appears unsuitable for diagnosing Candida infection.

Serum blocking factors, originally described in studies of tumour immunity, are thought to be antigen-antibody complexes (Sjögren *et al.*, 1971). If blocking factors in dermatophytosis are analogous, one would expect them to require a continual supply of antigen, as in chronic infection, and to be difficult to detect serologically.

A recent report (Hanifin, Ray & Lobitz, 1974) has described the detection of CMI in dermatophytosis by means of the lymphocyte transformation technique. Patients with either *T. mentagrophytes* or *T. rubrum* infections, who gave delayed skin reactions to the mixed antigen trichophytin, had lymphocytes which transformed equally well with antigen from either species during a 5-day incubation period *in vitro*. Lymphocytes from patients with chronic *T. rubrum* infection were non-reactive and these patients lacked delayed skin reactivity. Serum from such patients did not inhibit positive transformation responses. Although the antigens were prepared differently, the above results are so different from the ones obtained by the LAI test that one must infer that the two techniques are concerned with distinct phenomena. It may thus be misleading to suppose that a single technique can reveal all the facets of CMI, but for diagnostic purposes LAI has the advantages of rapidity, simplicity, species-specificity and ability to demonstrate serum blocking factors.

The observation that acute and chronic dermatophytoses can now be distinguished from each other by an *in vitro* test (Walters *et al.*, 1974) may be of value in prognosis and treatment. Whereas acute infections usually respond well to locally applied antifungal agents, chronic infections require more prolonged and extensive treatment. This is presumably because specific CMI is needed to supplement chemotherapy, which is ineffectual when the natural cellular defence is blocked. The finding of specific blocking factor in the serum is an objective index of chronicity and may dictate the use of griseofulvin in therapy but may also indicate the reason for failure of treatment with that drug.

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REFERENCES

CONANT, N.F., SMITH, D.T., BAKER, R.D. & CALLAWAY, J.L. (1971) *A Manual of Clinical Mycology*, 3rd edn. Saunders, Philadelphia.

DUNNE, M.R. (1962) A rapid method for the identification of dermatophytes. *Australian Journal of Dermatology*, 7, 294.

GRAPPEL, S.F., BISHOP, C.T. & BLANK, F. (1974) Immunology of dermatophytes and dermatophytosis. *Bacteriological Reviews*, **38**, 222.

HALLIDAY, W.J., MALUISH, ANNETTE & ISBISTER, W.H. (1974) Detection of anti-tumour cell-mediated immunity and serum blocking factors in cancer patients by the leukocyte adherence inhibition test. *British Journal of Cancer*, **29**, 31.

HALLIDAY, W.J. & MILLER, SUSAN (1972) Leukocyte adherence inhibition: a simple test for cell-mediated tumour immunity and serum blocking factors. *International Journal of Cancer*, **9**, 477.

HANIFIN, J.M., RAY, L.F. & LOBITZ, W.C., JR (1974) Immunological reactivity in dermatophytosis. *British Journal of Dermatology*, **90**, 1.

HAZEN, E.L., GORDON, M.A. & REID, F.C. (1970) *Laboratory Identification of Pathogenic Fungi Simplified*, 3rd edn. Charles C. Thomas, Springfield, Illinois.

JONES, H.E., REINHARDT, J.H. & RINALDI, M.G. (1973) A clinical, mycological and immunological survey for dermatophytosis. *Archives of Dermatology*, **108**, 61.

LONGBOTTOM, JOAN L., MURRAY, J.G. & PEPYS, J. (1968) In: *Clinical Aspects of Immunology* (Ed. by P.G.H. Gell and R.R.A. Coombs), 2nd edn, p. 71. Blackwell Scientific Publications, Oxford.

SJÖGREN, H.O., HELLSTRÖM, I., BANSAL, S.C. & HELLSTRÖM, K.E. (1971) Suggestive evidence that the 'blocking antibodies' of tumor-bearing individuals may be antigen–antibody complexes. *Proceedings of the National Academy of Sciences of the U.S.A.*, **68**, 1372.

WALTERS, B.A.J., CHICK, J.E.D. & HALLIDAY, W.J. (1974) Cell-mediated immunity and serum blocking factors in patients with chronic dermatophytic infections. *International Archives of Allergy and Applied Immunology*, **46**, 849.

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The leukocyte migration inhibition test in allergic nickel contact dermatitis

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SUMMARY

No statistical difference was found between the migration indices of nickel sensitive and control subjects using the leukocyte migration inhibition test with two concentrations of nickel sulphate. The results contrast with the specific positive results demonstrated previously in the same subjects with the lymphocyte transformation test.

In nickel sensitive subjects positive lymphocyte transformation tests are obtained when nickel sulphate or acetate is used in optimum concentration as a stimulant. This lymphocyte transformation is specific for subjects who show clinical and patch test evidence of nickel sensitivity (Hutchinson, Raffle & MacLeod, 1972). In cell-mediated immune reactions a number of humoral factors are elaborated by lymphocytes in contact with antigens. Of these, macrophage inhibition factor (MIF) production is regarded as a correlate of clinical delayed hypersensitivity states. The relationship between MIF and leukocyte migration inhibition (LMI) factor is not entirely clear but LMI is commonly employed in the assessment of clinical states of delayed hypersensitivity (Mitchell *et al.*, 1972). We have carried out LMI tests in nickel-sensitive patients to ascertain whether this test can be used to confirm clinical sensitivity.

PATIENTS AND METHODS

Ten subjects with clinical evidence of nickel dermatitis together with positive patch tests and positive lymphocyte transformation tests were studied. LMI tests were carried out by the method of Federlin *et al.* (1971) and Maini *et al.* (1973), the only modification being in the use of medium 199 instead of Waymouth medium. The nickel sensitive subjects were paired with control subjects for the study. Two different concentrations of nickel were employed (as sulphate) in the tests, 10^{-4} and 10^{-6} mEq Ni/ml of medium. In three cases and three controls peripheral blood lymphocytes were also cultured with nickel salts for 3 days prior to performing the LMI tests against independent effector cells on the basis that such prior incubation might be necessary to elaborate LMI in sufficient concentration to

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produce a positive test. In two subjects 25% autologous serum or 25% reconstituted freeze-dried plasma were substituted for 10% horse serum as an alternative to provide a potential protein conjugate for the nickel hapten.

RESULTS

There was no statistical difference between the migration indices of the nickel sensitive and control subjects for the two different concentrations of antigen used. The maximum variation was less than ±20% of unity. No consistent evidence of LMI factor elaboration under the conditions of these experiments was obtained.

DISCUSSION

These results are contrary to those obtained by Mirza, Perera & Bernstein (1974) who reported positive LMI tests in patients with proven nickel dermatitis. A negligible difference would be expected between the use of bovine albumin as a nickel albumin complex and the methods used in some of our tests, i.e. 10% horse serum added to the medium in Federlin *et al.*'s technique or the 25% autologous serum or reconstituted freeze-dried plasma. Hence the conflicting results may be due to a concentration effect. Positive LMI results have also been reported by Thulin & Zachariae (1972) in chromium sensitive subjects when chromium bound to bovine albumin was employed as the antigen, but de Jong, Bleumink & Nater (1974) have shown that positive LMI tests with mercuric chloride, a non-specific mitogen, resulted from a decrease in the number of migrating polymorphonuclear leukocytes, and hence were false positives. They demonstrated that a concentration of 3×10^{-5} M Hg induced lysis of 30–50% of the leukocytes within 30 min of incubation. Unlike the lymphocyte transformation test the LMI test is clearly of doubtful value in the diagnosis of delayed hypersensitivity to nickel in dermatitis subjects.

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REFERENCES

DE JONG, M.C.J.M., BLEUMINK, E. & NATER, J.P. (1974) *In vitro* leukocyte migration inhibition studies of allergic contact dermatitis in man. *9th European Congress of Allergology and Clinical Immunology* (in press).

FEDERLIN, K., MAINI, R.N., RUSSELL, A.S. & DUMONDE, D.C. (1971) A micro-method for peripheral leukocyte migration in tuberculin sensitivity. *Journal of Clinical Pathology*, **24**, 533.

HUTCHINSON, F., RAFFLE, E.J. & MACLEOD, T.M. (1972) The specificity of lymphocyte transformation *in vitro* by nickel salts in nickel sensitive subjects. *Journal of Investigative Dermatology*, **58**, 362.

MAINI, R.M., ROFFE, L.M., MAGRATH, I.T. & DUMONDE, D.C. (1973) Standardization of the leukocyte migration test. *International Archives of Allergy*, **45**, 308.

MIRZA, A.M., PERERA, M.G. & BERNSTEIN, I.L. (1974) Leukocyte migration inhibition in nickel dermatitis. *Federation Proceedings*, **33**, 728.

MITCHELL, CHRISTINE G., SMITH, M.G.M., GOLDING, P.L., EDDLESTON, A.L.W.F. & WILLIAMS, R. (1972) Evaluation of the leukocyte migration test as a measure of delayed hypersensitivity in man. *Clinical and Experimental Immunology*, **11**, 535.

THULIN, H. & ZACHARIAE, H. (1972) The leukocyte migration test in chromium hypersensitivity. *Journal of Investigative Dermatology*, **58**, 55.

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A study on the clinical application of a direct leukocyte migration test in chromium contact allergy

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SUMMARY

A capillary tube leukocyte migration inhibition assay has been adopted as an *in vitro* method for the demonstration of chromium hypersensitivity in twenty-two subjects with clinically proven or suspected chromium allergy. Two complexes of trivalent chromium and human serum albumin, exerting different migration inhibitory effects, have been prepared and used as the antigens.

In the presence of the chromium–albumin complex with strong inhibitory activity, sensitivity to chromium was demonstrated independent of the clinical condition of the skin in all the patch test positive subjects. An additional positive response to the second chromium–albumin complex was observed only in those patients who were clinically in a state of exacerbation of an allergic contact dermatitis in which chromium allergy was an active causative factor.

The results were not influenced by skin allergic reactivity to compounds other than chromium and the method was found to be of practical clinical value for diagnosing chromium allergy.

Leukocyte migration tests (LMT) have found wide employment in immunopathology and denote delayed type immunity in man. They have proved effective in bacterial (Søborg, 1967; Clausen & Søborg, 1969; Clausen, 1970; Budtz-Jørgensen, 1972; Godal *et al.*, 1972; Rauch & King, 1973) and parasitic (Gaines *et al.*, 1971) infections, in auto-allergic diseases (Søborg & Halberg, 1968; Brostoff & Walker, 1971) and in certain contact allergic skin disorders (Nordqvist & Rorsman, 1967; Herman & Sams, 1971). Their application in investigative dermatology, however, has been limited by the difficulties often met in finding and preparing the proper antigens.

Chromium allergy is a common cause of contact eczema and has been the subject of extensive study by many investigators. *In vitro* sensitivity to chromium has been demonstrated by Thulin & Zachariae (1972) using hexavalent and trivalent chromium bound to bovine albumin as antigens.

The present paper reports the employment of complexes of trivalent chromium and human albumin as antigens in a practical application of Søborg & Bendixen's (1967) modification of the LMT in clinical allergy.

MATERIALS AND METHODS

The study was carried out on five healthy controls, nine patients with clinically proved and thirteen patients with suspected chromium allergic contact dermatitis.

In cases of active eczema routine patch tests were performed as soon as the skin condition allowed such a procedure. Patients with a clinical record of chromium allergy but free of symptoms for at least 3 months were not subjected to epicutaneous testing.

Antigen preparation

Two stock solutions of chromium chloride and human albumin were prepared as follows:

(A) Five hundred mg of human albumin (Fraction V, Sigma) and 250 mg of chromium chloride ($CrCl_3.6H_2O$, Merck) were dissolved in 15 ml of distilled water. The solution was stored overnight at 4°C and dialysed against distilled water for 72 h.

(B) The same amounts of human albumin and chromium chloride were dissolved in 7·5 ml of distilled water and stored overnight at 4°C. Prior to dialysis the volume was adjusted to 15 ml with distilled water and processed as mentioned above.

After dialysis, protein concentrations were estimated by Lowry's method (1951) and chromium contents as described by Cohen (1966a).

Solution A consisted of 1·95 g% human albumin (HuA) and 1 g% chromium (Cr). Ten, 20, 40, and 50 μl/ml medium, corresponding with 0·1 mg Cr/0·195 mg HuA, 0·2 mg Cr/0·390 mg HuA, 0·4 mg Cr/0·780 mg HuA, and 0·5 mg Cr/0·975 mg HuA per ml medium respectively, were used for testing.

Solution B consisted of 1·8 g% human albumin (HuA) and 0·82 g% chromium (Cr). Preliminary studies with concentrations ranging from 2–10 μl/ml have established that 2 and 4 μl/ml, corresponding with 0·016 mg Cr/0·036 mg HuA and 0·032 mg Cr/0·072 mg HuA per ml, gave the most consistent results. At the higher concentrations heavy precipitate formation was observed.

Stock solutions were sterilized by millipore filtration (pore size 0·45 μm) and stored at 4°C. Test solutions were prepared and pH adjusted to 7·3 shortly before setting up cultures.

Leukocyte migration test

The technique described by Søborg & Bendixen (1967) was followed, but with a few modifications. Twenty ml of blood was taken by venepuncture in sterile disposable plastic syringes containing preservative-free heparin (Thromboliquine, Organon) at a final concentration of 20 i.u./ml. White blood cells were isolated by Dextran–Isopaque sedimentation as described by Böyum (1968), and washed three times by serial centrifugation in Earle's balanced salt solution. The final cell concentration was adjusted to approximately 30×10^6/ml and the cell suspension aspirated into microcapillary tubes (10 μl volume, Vitrex, Chr. Bardram). One end of each tube was sealed with sealing wax (D553, Radiometer Copenhagen) followed by centrifugation at 400 *g* for 5 min. The tubes were then cut 1 mm above the cell fluid interphase and the cell-containing parts of two tubes fixed in a tissue culture chamber (1 ml volume) with small dots of silicone grease (Dow Corning). Control cultures were filled with Minimal Essential Medium (Earle's salts), supplemented with 20% heat inactivated newborn calf serum, 20 mM Hepes buffer, 20 mM L-glutamine (all from Flow Amstelstad), gentamicin 50 μg/ml (Garamycine Schering U.S.A.), pH 7·3.

Test cultures were filled with medium containing the antigens. Cultures were incubated for 20 h at 37°C and the area over which the cells had migrated was projected (Leitz projector) and traced on paper. Whole migration areas were measured planimetrically. From the average of 4 migration areas of test cultures and 4 migration areas of controls the migration index was calculated as follows:

$$\text{M.I.} = \frac{\text{migration in the presence of antigen}}{\text{migration in medium alone}}$$

Statistical analysis was performed by means of the Student's '*t*'-test on the differences of paired observations.

Routine patch tests were performed according to the International Contact Dermatitis Research Group recommendations. Readings were done after 48 and 72 h.

RESULTS

According to clinical records and results of patch tests performed afterwards, LMT have been performed in five healthy controls, five chromium negative and seventeen chromium positive persons. The normal ranges of migration indices (M.I.), observed in the healthy controls and the chromium negative persons in the presence of various doses of the solutions A and B, are given in Table 1. As shown in Table 2, a distinct separation between chromium negative and chromium positive persons could be obtained with 40 μl/ml of solution A ($P<0\cdot001$). However, the clinical significance of this finding has been reduced by the qualitative aspects of the results and the lack of correlation with the clinical pictures of the skin disorders.

TABLE 1. Clinical aspects, migration indices and positive patch test results to other agents in five controls and five chromium negative subjects

Clinical aspect	Other agents	Doses (μl/ml)					
		Solution A				Solution B	
		10	20	40	50	2	4
Healthy control		0·98	1·00	0·92	0·96	0·89	0·95
Healthy control		0·97	0·94	0·94	0·99	0·97	0·97
Healthy control		0·94	0·90	0·86	0·90	0·92	0·95
Healthy control		0·95	0·89	0·83	0·84	0·98	0·88
Healthy control		0·87	0·85	0·93	0·88	0·95	0·84
Hand eczema			0·97	0·88	0·86		
Hand eczema						0·86	0·88
Hand eczema						0·85	0·91
Active disseminated eczema	p-phenylenediamine aniline	0·91	0·86	0·83	0·84	0·86	1·00
Active disseminated eczema	Epoxy resin		1·00	1·00		0·87	0·86

A different type of response was observed in the presence of 4 μl/ml of solution B. Specific inhibition of migration ($P<0\cdot005$) was elicited only in those patients (Nos 11–17) exhibiting the clinical symptoms of an allergic contact dermatitis in which chromium allergy was an active causative factor. It is of note that patients Nos 16 and 17 have also been tested during a symptom-free period (Nos 1 and 2, respectively). Consequently, chromium allergic persons without the clinical signs of an active skin disorder (Nos 1–7) and those exhibiting active skin lesions due to other contact sensitizing agents (Nos 9 and 10) or due to other factors (No. 8) were found to be unresponsive to the challenge with solution B.

The results of the LMT performed in the presence of 40 μl/ml of solution A and 4 μl/ml of solution B are summarized in Fig. 1.

TABLE 2. Clinical aspects, migration indices and positive patch test results to other agents in seventeen chromium positive subjects

			Doses (µl/ml)					
			Solution A				Solution B	
No.	Clinical aspects	Other agents	10	20	40	50	2	4
1	Symptom free	Nickel, cobalt		0·78	0·65	0·60	1·05	1·05
2	Symptom free	Nickel, cobalt, formaldehyde	0·86	0·60			0·95	0·83
3	Inactive	Nickel, cobalt, benzocaine, t.m.t.d., thiuram		0·87	0·53		0·91	0·82
4	Inactive	Nickel, cobalt	0·85	0·75	0·70		1·05	0·91
5	Symptom free	—	0·86	0·83	0·76	0·60		
6	Symptom free	—		0·86	0·74	0·74		
7	Inactive	—		0·76	0·79	0·70		
8	Toxicoderma	Cobalt	0·97	0·79	0·72	0·68	0·91	1·00
9	Active disseminated	Cobalt, formaldehyde	0·86	0·74	0·66	0·77	0·81	0·84
10	Active generalized	Cobalt, nickel, chrysanthemum	0·90	0·98	0·59	0·67	0·98	1·01
11	Active localized	—					0·85	0·64
12	Active localized	—	0·61	0·55	0·50		0·63	0·57
13	Active disseminated	Cobalt	0·65	0·63			0·76	0·47
14	Active disseminated	Nickel, cobalt	0·80	0·70	0·62	0·64	0·80	0·67
15	Active disseminated	Thiuram, Carbamix, perubalsam, benzoyl peroxide	0·94	0·76	0·71		1·05	0·71
16	Active localized	Nickel, cobalt					0·72	0·54
17	Active localized	Nickel, cobalt, formaldehyde					0·75	0·52

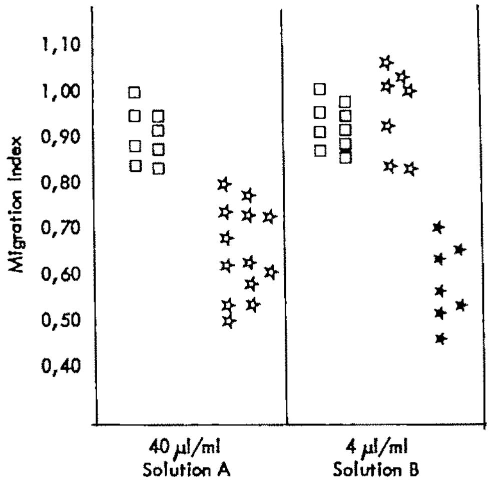

FIGURE 1. The effect of 40 µl/ml of solution A and 4 µl/ml of solution B on the leukocyte migration indices of chromium negative (squares) and chromium positive (stars) persons. Open stars, chromium positive without symptoms or with allergic contact dermatitis due to other compounds than chromium. Filled stars, chromium positive with active chromium allergic contact dermatitis.

DISCUSSION

The data presented show that inhibition of migration of autologous leukocytes induced by trivalent chromium-human albumin is a sensitive *in vitro* parameter of chromium hypersensitivity. It is also shown that the assay may be used for different purposes. If the most important objective is to distinguish between chromium negative and chromium positive persons, false negative results can be avoided by using the appropriate dose of the solution A. On the other hand it may be of practical importance to know whether chromium compounds or other agents are actively involved in the eruptive stage of an allergic contact dermatitis, in which case solution B should be employed.

As suggested in Fig. 1, a quantitative estimation of the migration inhibition reaction may be obtained by performing LMT with only two test doses, the 40 μl/ml of solution A and the 4 μl/ml of solution B.

Results obtained so far seem to indicate that the principle of the method is applicable in chromium allergy and may be with other common contact allergens as well.

Our results with trivalent chromium compound are in agreement with the latest concepts on the 'true' nature of the hapten in chromium hypersensitivity, as suggested by Cohen (1966a), Samitz *et al.* (1969), Polak & Frey (1973) and Shmunes, Katz & Samitz (1973).

The difference in the migration inhibitory activity of the two test solutions is not easily explained. Since human albumin has been used as the carrier, structural changes of the protein in the presence of the chromium salt (Clark, 1959) might have influenced the physico-chemical properties of the complex. Consequently, the different procedure adopted for the preparation of solutions A and B might have resulted in the formation of two different complexes with different antigenic activity. On the other hand, the nature of the carrier may be of less importance (Cohen, 1966b, 1968) and the difference in the migration inhibitory activity might as well be attributed to the different amounts of chromium ions present in the cultures. In that case other factors, such as the presence of a specific antigen recognizing system on the cells participating in the LMT as well as the direct action of the antigens on the migrating cell population, must be kept in mind and need further evaluation.

Despite the lack of detailed knowledge of the true mechanism underlying the assay, LMT has by virtue of its sensitivity become a valuable tool for fundamental and experimental research in biology and medicine. The results of this study suggest that LMT may be adopted for practical purposes in clinical dermatology as well.

REFERENCES

Böyum, A. (1968) Separation of leukocytes from blood and bone marrow. *Scandinavian Journal of Clinical and Laboratory Investigation*, **97**, 1.

Brostoff, J. & Walker, J.G. (1971) Leukocyte migration inhibition with Kveim antigen in Crohn's disease. *Clinical and Experimental Immunology*, **9**, 707.

Budtz-Jørgensen, E. (1972) Delayed type hypersensitivity to candida albicans in man demonstrated *in vitro:* the capillary tube migration test. *Acta Allergologica*, **27**, 41.

Clark, J.H. (1959) The denaturation of proteins by chromium salts. *Archives of Industrial Health*, **20**, 117.

Clausen, J.E. (1970) Polymorphonuclear leukocytes in the specific antigen-induced inhibition of the *in vitro* migration of human peripheral leukocytes. *Acta Medica Scandinavica*, **188**, 59.

Clausen, J.E. & Søborg, M. (1969) *In vitro* detection of tuberculin hypersensitivity in man. Specific migration inhibition of white blood cells from tuberculin-positive persons. *Acta Medica Scandinavica*, **186**, 227.

Cohen, H.A. (1966a) Carrier specificity of tuberculin-type reaction to trivalent chromium. *Archives of Dermatology*, **93**, 34.

Cohen, H.A. (1966b) Tuberculin-type reaction to heparin–chromium complex. *Archives of Dermatology*, **94**, 409.

Cohen, H.A. (1968) Hyaluronic acid. A specific carrier of chromium sensitivity. *Archives of Dermatology*, **98**, 148.

GAINES, J.D., ARAUJO, F.G., KRAHENBUHL, J.L. & REMONGTON, J.S. (1971) Simplified *in vitro* method for measuring delayed type hypersensitivity to latent intracellular infection in man (toxoplasmosis). *Journal of Immunology*, **107**, 906.

GODAL, T., MYRVANG, B., FROLAND, S.S., SHAO, J. & MELAKU, G. (1972) Evidence that the mechanism of immunological tolerance ('central failure') is operative in the lack of host resistance in lepromatous leprosy. *Scandinavian Journal of Immunology*, **1**, 311.

HERMAN, P.S. & SAMS, W.M. JR (1971) Requirement for carrier protein in salicylanilide sensitivity: the migration inhibition test in contact photo allergy. *Journal of Laboratory and Clinical Medicine*, **77**, 572.

LOWRY, O.H., ROSENBROUGH, N.J., FARR, A. & RANDALL, R.J. (1951) Protein measurement with folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265.

NORDQVIST, B. & RORSMAN, H. (1967) Leukocyte migration *in vitro* as an indicator of allergy in eczematous contact dermatitis. *Transactions of the St John's Hospital Dermatological Society* (*London*), **53**, 154.

POLAK, J. & FREY, J.R. (1973) Studies on contact hypersensitivity to chromium in the guinea-pig. *International Archives of Allergy*, **44**, 51.

RAUCH, H.C. & KING, K. (1973) Human leukocyte migration inhibition as an indicator of cellular hypersensitivity to soluble antigens. *International Archives of Allergy*, **44**, 862.

SAMITZ, M.H., KATZ, S.A., SCHNEIDER, D.M. & GROSS, P.R. (1969) Chromium–protein interactions. *Acta dermato-venereologica*, **49**, 142.

SHMUNES, E., KATZ, S.A. & SAMITZ, M.H. (1973) Chromium–amino acid conjugates as elicitors in chromium sensitized guinea-pigs. *Journal of Investigative Dermatology*, **60**, 193.

SØBORG, M. (1967) *In vitro* detection of cellular hypersensitivity in man. Specific migration inhibition of white blood cells from brucella positive persons. *Acta Medica Scandinavica*, **182**, 167.

SØBORG, M. & BENDIXEN, G. (1967) Human lymphocyte migration as a parameter of hypersensitivity. *Acta Medica Scandinavica*, **181**, 247.

SØBORG, M. & HALBERG, P. (1968) Cellular hypersensitivity in Hashimoto's thyroiditis. Specific action of thyroid extract upon the migration of leucocytes '*in vitro*'. *Acta Medica Scandinavica*, **183**, 101.

THULIN, H. & ZACHARIAE, H. (1972) The leucocyte migration test in chromium hypersensitivity. *Journal of Investigative Dermatology*, **58**, 55.

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Pharmacology and Treatment

An assessment of high and low temperature tars in psoriasis

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SUMMARY

Comparison of the efficacy of crude coal tar from high and low temperature sources in the treatment of patients suffering from chronic psoriasis showed the tars to be equally effective.

High temperature tar was then compared with standard refined tar. Again, an equal therapeutic response was achieved.

Crude coal tars obtained by the carbonization of coal in coke ovens and in smokeless fuel manufacture can be employed in dermatological therapy in place of the dwindling supplies of crude tar of gasworks origin.

While many papers have been published over the past 2 decades on the use of topical corticosteroid preparations in dermatology, very little has been said about the traditional tar that has been employed for so many years (Young, 1970; Grupper, 1971). Many centres still find tar invaluable for the control of chronic dermatoses and especially for the treatment of psoriasis. Unfortunately, gasworks which have been the main source of crude tar in the United Kingdom have been progressively shut down as natural gas supplies are utilized from the North Sea and elsewhere. In consequence, pharmacists are now finding difficulty in obtaining crude coal tar for dermatological use.

Although coal tar obtained from vertical retorts at gasworks has been usually regarded as a high temperature tar, it in fact possesses characteristics intermediate between those of high and low temperature tars (Cislak, 1955). A high temperature tar is formed during the carbonization of coal to 900–1200°C as occurs in coke ovens in steel manufacture. A low temperature tar (Pratt & Cislak, 1955) is produced by carbonization of bituminous coals to temperatures between 450 and 700°C as in smokeless fuel manufacture.

In view of further supplies of gasworks tar being unobtainable, we undertook comparison of a high temperature tar of coke oven origin with a low temperature tar produced during smokeless fuel manufacture. Each tar was representative of its type. Comparison was later made with the tar employed at present in our hospital. This is conventional crude coal tar of gasworks origin which has been distilled to remove volatile materials.

METHODS

The hospital pharmacy arranged for the high and low temperature crude coal tars to be made up in ointment form in an identical fashion to our standard refined tar. Concentrations of tar were 2, 4, 6 and 10% in zinc oxide 2 parts, starch 25 parts, soft paraffin to 100; 20% tar was contained in a base composed of zinc oxide 20 parts, lanolin 30 parts, vaseline 30 parts.

In-patients admitted to the trial were suffering from stable symmetrical chronic psoriasis. Tar ointments were applied twice daily to affected areas but ultraviolet light therapy and tar baths were omitted. Each patient had a conventional daily bath during which residual ointment and scales were rubbed off. Tar concentrations were increased steadily according to the patient's progress and tolerance.

RESULTS

The tars were compared under double-blind conditions, the key to their identities being held by the pharmacy. One tar was used on an arm and a leg on the same side while the other tar was used on the opposite limbs. The ointments were allocated on a random left/right basis. Standard refined tar was applied twice daily to affected areas on the trunk.

Each week, comparison of progress on the two sides was made by examination of plaque thickness, erythema and scaling. A final comparison was made at 3 weeks. Note was also made of undue staining of the skin and of complications such as irritation, folliculitis and boils.

Of twenty-two patients in whom comparison was made, four were found to respond better to the high temperature tar, three better to the low temperature tar and in fifteen there was no apparent difference.

In the seven patients where a difference was noted, it was of mild degree.

Four patients each developed a boil, two on skin treated with high temperature tar and two on skin treated with low temperature tar. Mild bilateral folliculitis was observed in two patients while in another the folliculitis was localized to a limb treated with low temperature tar. One individual experienced temporary irritation with both tars, but more so with the low temperature preparation. The remaining fourteen patients had no complications.

It would, therefore, appear that high and low temperature crude coal tars are equally effective in the treatment of chronic psoriasis. Both tars resulted in folliculitis in a number of patients. The incidence appeared acceptable and compatible with past experience of tar treatment. Eight of the twenty-two patients showed deep staining of the treated skin following use of high temperature tar while only two showed this feature on areas treated with low temperature tar.

Comparison of high temperature tar with standard refined tar

Since Downing & Baer (1948) had found a high temperature tar to be the most effective of all those they investigated, we next undertook a comparison of our standard refined tar with the high temperature tar. The trial was conducted in the same way as the previous investigation except that it was not conducted in a double-blind fashion owing to the staining produced by the high temperature tar. The efficacy of both tars was compared over a 3-week period, in patients suffering from chronic psoriasis.

Of fourteen patients, three obtained more effective results with the high temperature tar, two made better progress with the standard tar and nine showed equal improvement with the two preparations. Where an advantage was observed for one or other tar, it was again very slight just as in the previous investigation. It was noteworthy how, in the course of each comparison, the side treated with the crude tar responded more quickly in the earlier stages of treatment, only for the other side to catch up towards the end of the 3-week period.

Mild folliculitis of equal degree on each side occurred in two patients while in another two patients the folliculitis was confined to the areas being treated with high temperature tar. The remaining ten cases showed no complications.

DISCUSSION

It would appear from the similarity of our results obtained in psoriasis using a high and a low temperature tar that these preparations influence the psoriatic process equally effectively. At first glance, this is surprising in view of the great differences in composition of the tars. There is suggestive recent evidence, however, from the work of Wrench & Britten (1975 a, b), in which they employed the mouse tail skin as an experimental model, that the active anti-psoriatic constituents are to be found in the phenols or high boiling tar acids of the creosote and anthracene oil fractions of crude tar. Wrench & Britten found that granular layer induction was produced mainly by the high boiling tar acids in the range 280–340°C, peak induction being obtained with the fraction boiling at 330–340°C (Wrench & Britten, 1975c). Such high boiling tar acids contain many substituted polyhydroxyphenols and their isomers related to dithranol (Coal Tar Data Book, 1970). The tar acid content of a low temperature tar may attain 15–18% while the total tar acid content of a high temperature tar is around 5%. Their presence would explain the effectiveness of crude coal tar preparations and also how their removal in tar refining gives a less effective end-product. Wrench & Britten (1975a) also make the interesting point that skin staining was produced mainly by the phenolic compounds that they found showed most therapeutic promise.

Past clinical experience gives supportive evidence for Wrench & Britten's work. Kinmont (1957) quotes how the dispenser at Jadassohn's clinic was instructed to obtain his tar supply from the depths of the barrel, presumably to give a higher content of middle and heavy oils, naphthalene and anthracene oils.

The contribution of tar phenols to therapeutic activity of tar was discussed by Hellier & Whitefield (1967) while Thorne (1963) found that a mixture of tar acids and bases gave better results in psoriasis than crude coal tar. Too much, however, should not be deduced from Wrench & Britten's work since they found that dithranol, a very effective agent in psoriasis, did not cause induction of a granular layer in mouse tail skin (Wrench & Britten, 1975 d). In 1948, for instance, Downing & Baer assessed the therapeutic effectiveness of high and low temperature tars in hundreds of patients with eczema and psoriasis. Although no single tar was found to be best for all purposes, two of the high temperature tars were found to give the best results, although their content of high boiling tar acids would be less than in a low temperature tar.

Our study in a small group of patients suggests that a high temperature and a low temperature crude coal tar have equivalent therapeutic effects in patients with chronic psoriasis. Comparison of the high temperature tar with refined gasworks tar again gave equally good clinical improvement. It seems a reasonable conclusion that crude coal tar of high or low temperature derivation can be used successfully in the treatment of psoriasis. Until more specific fractions of tar are available, dermatologists will still be able to use crude coal tars of these types in their therapeutic armoury.

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REFERENCES

CISLAK, F.E. (1955) Coal Tar. In: *Encyclopaedia Britannica*, Vol. 5, p. 883. Encyclopaedia Britannica Ltd., London.

COAL TAR DATA BOOK (1970) 2nd edn, Coal Tar Research Association, Cleckheaton, England.

DOWNING, J.G. & BAER, C.W. (1948) Low and high temperature coal tars in the treatment of eczema and psoriasis. *Archives of Dermatology and Syphilology*, **57**, 985.

GRUPPER, C. (1971) The chemistry, pharmacology and use of tar in the treatment of psoriasis. In: *Psoriasis, proceedings of the international symposium* (Ed. by E.M.Farber and A.J.Cox), p. 347. Stanford University Press.

HELLIER, F.F. & WHITEFIELD, M. (1967) The treatment of psoriasis with triacetoxyanthracene. *British Journal of Dermatology*, **79**, 491.

KINMONT, P.D.C. (1957) Tar and the skin. *The Practitioner*, **179**, 598.

PRATT, D.D. & CISLAK, F.E. (1955) Low temperature tars. In: *Encyclopaedia Britannica*, Vol. 21, p. 820. Encyclopaedia Britannica Ltd., London.

THORNE, N. (1963) The treatment of psoriasis with fractionated tar and lecithin. *British Journal of Dermatology*, **75**, 422.

WRENCH, R. & BRITTEN, A.Z. (1975a) Evaluation of coal tar fractions for use in psoriasiform diseases using the mouse tail test. I. High and low temperature tars and their constituents. *British Journal of Dermatology*, **92**, 569.

WRENCH, R. & BRITTEN, A.Z. (1975b) Evaluation of coal tar fractions for use in psoriasiform diseases using the mouse tail test. II. Tar oil acids. *British Journal of Dermatology*, **92**, 575.

WRENCH, R. & BRITTEN, A.Z. (1975c) Evaluation of coal tar fractions for use in psoriasiform diseases using the mouse tail test. III. High boiling tar oil acids. *British Journal of Dermatology*, **93**, 67.

WRENCH, R. & BRITTEN, A.Z. (1975d) Evaluation of dithranol and a 'synthetic tar' as anti-psoriatic treatments using the mouse tail test. *British Journal of Dermatology*, **93**, 75.

YOUNG, E. (1970) The external treatment of psoriasis. *British Journal of Dermatology*, **82**, 510.

British Journal of Dermatology (1976) 94, 75.

Treatment of erythema nodosum and nodular vasculitis with potassium iodide

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SUMMARY

Twenty-four of twenty-eight patients with erythema nodosum and sixteen of seventeen patients with nodular vasculitis responded to treatment with potassium iodide. Relief of symptoms occurred within 2 days. The average duration of treatment was 3 weeks and the lesions took an average of 2 weeks to resolve. The possible mode of action of potassium iodide is discussed and an immunosuppressive effect mediated by heparin is suggested.

Treatment of erythema nodosum and nodular vasculitis with anti-inflammatory drugs such as salicylates, indomethacin and corticosteroids often gives unsatisfactory results. Although potassium iodide was formerly used for erythema nodosum it has long been out of fashion. Reports of the excellent results obtained with potassium iodide in the treatment of subacute nodular migratory panniculitis (Vilanova & Aguadé, 1956, 1959; Perry & Winkelmann, 1964) prompted us to try it in cases of erythema nodosum and nodular vasculitis.

PATIENTS AND METHODS

Forty-five patients with nodular inflammatory lesions on the lower legs were included in this study. The lesions consisted of one to many nodules or plaques situated mainly over the shins, although the calf was involved in addition in some patients. None of the lesions was ulcerated. Twenty-two of the patients had had previous treatment including salicylates, oxyphenbutazone, phenylbutazone, indomethacin, sulphonamides, trimethoprim and corticosteroids without appreciable effect. Routine investigations included full blood count and sedimentation rate, culture of throat swab, antistreptolysin O titre, Yersinia agglutination test and X-ray examination of the chest, sinuses and teeth. Skin biopsies were done in addition in twelve patients. The patients were divided into two groups as follows:

(a) *Erythema nodosum*—twenty-eight cases. The ages of the patients ranged from 16 to 58 (median 29) years and all except one were female. The duration of the lesions before the patients were referred

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to us ranged from 1 to 8 (average 4·3) weeks. In seven patients the cause of the erythema nodosum was ascribed to tonsillitis, in seven to influenza, in four to sarcoidosis, in two to pregnancy, in two to oral contraceptives, in two to dental sepsis and in one to sinusitis. In two patients there was no obvious cause for the erythema nodosum.

(b) *Nodular vasculitis*—seventeen cases. The ages of the patients ranged from 17 to 56 (median 27) years. All except one were female. The duration of the lesions ranged from 8 to 52 (average 20·6) weeks. Seven of the patients had a history of recurrent attacks. In three patients influenza may have precipitated the attack. Sinusitis with fluid levels on X-ray examination was found in two patients and two had chronic tonsillitis. Dental infection may have played a role in one patient and oral contraceptives in another. In eight patients no possible cause was found.

Potassium iodide was given either in a mixture which is bitter, or as a powder dispensed in gelatin capsules to make it palatable. The daily dose varied from 360 to 900 mg and the duration of treatment from 3 days to 8 weeks. As relapses occurred in two patients treated for only 2 weeks early on in the trial, a treatment period of at least 3–4 weeks became routine. The maximum daily dose of potassium iodide was 900 mg in thirty-six patients, 540 mg in five and 360 mg in four patients. Pregnant patients were given the smaller doses and potassium iodide was not given during the last trimester to avoid suppression of fetal thyroid function. In five patients with tonsillitis a short course of antibiotics was given as well.

RESULTS

Forty of the forty-five patients responded to potassium iodide therapy. Marked decrease in pain and swelling occurred within 2 days. Complete resolution of lesions took an average of 2 weeks, with some of the larger plaques requiring up to 4 weeks to disappear completely. We did not attempt to establish a minimal effective dose for potassium iodide. However, in one patient the lesions relapsed on a dose of 540 mg daily but cleared completely on 900 mg daily. Side effects were encountered in six patients and consisted of nasal catarrh in four and headache and giddiness in two.

Five patients did not benefit from treatment with potassium iodide. In two with erythema nodosum the drug was stopped within 4 days because of side effects. A patient with nodular vasculitis of 16 weeks duration, who had fluid levels on X-rays of the sinuses, did not respond to 900 mg potassium iodide given for 3 weeks but the lesions cleared after a sinus washout. In the last two patients failure to respond may have been due to inadequate dosage. A pregnant woman with erythema nodosum showed no improvement while on 540 mg daily for 3 weeks and a patient with erythema nodosum of unknown cause took 540 mg daily for 2 weeks without response.

Subsequent recurrences in seven patients who responded initially to treatment with potassium iodide were treated in the same way with the same prompt improvement. One of these patients, a woman of 27 years when first treated for nodular vasculitis, has had recurrent attacks almost monthly for 8 years. The lesions are controlled with 600 mg potassium iodide daily, taken for a few days at a time.

DISCUSSION

In the early part of this century iodine was one of the drugs prescribed for erythema nodosum although this use was not generally accepted by the medical profession. While Brocq recommended it, Darier felt that it was contraindicated (Tachau, 1928). Potassium iodide was reported to be very effective in the treatment of subacute nodular migratory panniculitis by Vilanova & Aguadé (1956,

1959) and Perry & Winkelmann (1964) but less success was obtained in small numbers of patients with so-called chronic forms of erythema nodosum (Fine & Meltzer, 1969; Förström & Winkelmann, 1975). We have found potassium iodide very effective in the symptomatic treatment of both classical erythema nodosum and the more chronic recurrent nodular lesions of the lower leg which may be better classified as nodular vasculitis (Ryan & Wilkinson, 1972). In our experience iodide gave more consistent results than systemic corticosteroids, oxyphenbutazone and indomethacin. A dose of 900 mg potassium iodide given daily for 2–4 weeks was sufficient to control most cases. A feature of the response was the rapidity with which pain and swelling were ameliorated. As this trial was uncontrolled and the duration of nodular vasculitis is unpredictable, we cannot conclude that treatment with potassium iodide shortened the natural course of the diseases. The rapid symptomatic improvement however obviated the need for further treatment such as bandaging and bed rest.

The non-endocrine effects of iodides have recently been summarized by Stone (1971). They have a marked leukotactic effect and enhance the proteolytic, phagocytic and bactericidal action of polymorphonuclear leukocytes. Iodides are bactericidal when acting in conjunction with granulocyte myeloperoxidase (Klebanoff, 1967) but in syphilis and sporotrichosis they affect the tissue response and not the causative organism (Cushny, 1941). Iodides intensify the acute inflammatory response in experimental animals (Stone & Willis, 1967) but inhibit granuloma formation, perhaps by lessening fibrosis (Mielens, Rozitis & Sansone, 1968). Iodides have been shown to concentrate in infected granulomatous and necrotic tissue in experimental animals (Sternberg *et al.*, 1955). Potassium iodide is not a general anti-inflammatory agent as it inhibits only one model of inflammation in experimental animals. This action is independent of any effect on the thyroid gland (Mielens *et al.*, 1968).

Autoradiographic studies have demonstrated that iodides concentrate in mast cells (Jones, Buse & Worthington, 1964) and they have been shown to cause mast cell degranulation in rats (Cantin, 1967). Polymorphs seem to be necessary for the degranulation of mast cells (Gryfe, Sanders & Gardner, 1971) and the leukotactic effect of iodides may therefore enhance the reaction. Heparin, which is released in large quantities from mast cell granules, has been shown to inhibit delayed hypersensitivity reactions (Cohen *et al.*, 1967).

Acute, classical erythema nodosum and the more chronic nodular diseases of the lower leg such as nodular vasculitis show a spectrum of histological changes ranging from acute vasculitis with leukocytoclasis to tuberculoid granuloma formation, presenting features of both an Arthus and a delayed hypersensitivity reaction (Fine & Meltzer, 1969). Serological studies indicate that there is an overproduction of the mediators of cellular immunity in erythema nodosum (Krueger *et al.*, 1973). In summary, therefore, potassium iodide concentrates in granulomas and causes heparin release from mast cells. Heparin, in turn has been shown to suppress delayed hypersensitivity reactions which are implicated in the lesions of erythema nodosum and nodular vasculitis. The anti-inflammatory effect of potassium iodide in these conditions may be due to an immunosuppressive effect mediated by heparin.

Since formulating this theory we have treated four patients with a heparinoid ointment under plastic occlusion. In three (two with nodular vasculitis and one with erythema nodosum) the response paralleled that expected of potassium iodide, with dramatic improvement within 2 days and gradually clearing over 2–3 weeks. In a fourth patient with nodular vasculitis there was no improvement after 2 weeks of treatment with the heparinoid ointment and subsequently no response to systemic potassium iodide either. These results seem to confirm our theory that the effect of potassium iodide is mediated by heparin, and suggest that controlled trials using potassium iodide and heparinoid ointments in cases of erythema nodosum and nodular vasculitis may be warranted.

REFERENCES

CANTIN, M. (1967) Study of a generalized erythema induced by iodides. *Journal of Investigative Dermatology*, **48**, 560.

COHEN, S., BENACERRAF, B., MCCLUSKEY, R.T. & OVARY, Z. (1967) Effect of anticoagulants on delayed hypersensitivity reactions. *Journal of Immunology*, **98**, 351.

CUSHNY, A.R. (1941) The action of inorganic substances. In: *Pharmacology and Therapeutics* (Ed. by C.W. Edmunds and J.A. Gunn), 12th edn, p. 77. Churchill Ltd. London.

FINE, R.M. & MELTZER, H.D. (1969) Chronic erythema nodosum. *Archives of Dermatology*, **100**, 33.

FÖRSTRÖM, L. & WINKELMANN, R.K. (1975) Granulomatous panniculitis in erythema nodosum. *Archives of Dermatology*, **111**, 335.

GRYFE, A., SANDERS, P.M. & GARDNER, D.L. (1971) The mast cell in early rat adjuvant arthritis. *Annals of the Rheumatic Diseases*, **30**, 24.

JONES, D.J., BUSE, M.G. & WORTHINGTON, W.C. (1964) Mast cell localization of radioiodine in the rat. *Journal of Histochemistry and Cytochemistry*, **12**, 856.

KLEBANOFH, S.J. (1967) Iodination of bacteria: a bactericidal mechanism. *Journal of Experimental Medicine*, **126**, 1063.

KRUEGER, G.G., WESTON, W.L., THORNE, E.G., MANDEL, M.J. & JACOBS, R.J. (1973) A phenomenon of macrophage aggregation activity in sera of patients with exfoliative erythroderma, erythema multiforme and erythema nodosum. *Journal of Investigative Dermatology*, **60**, 282.

MIELENS, Z.E., ROZITIS, J. & SANSONE, V.J. (1968) The effect of oral iodides on inflammation. *Texas Reports on Biology and Medicine*, **26**, 117.

PERRY, H.O. & WINKELMANN, R.K. (1964) Subacute nodular migratory panniculitis. *Archives of Dermatology*, **89**, 170.

RYAN, T.J. & WILKINSON, D.S. (1972) Cutaneous vasculitis ('Angiitis'). In: *Textbook of Dermatology* (Ed. by A.J. Rook, D.S. Wilkinson and F.J. Ebling), 2nd edn. Vol. 1, p. 959. Blackwell Scientific Publications, Oxford.

STERNBERG, T.H., NEWCOMER, V.D., STEFFEN, C.G., FIELDS, M. & LIBBY, R.L. (1955) The distribution of radioactive iodide (I-131) in experimental coccidiodomycosis and sporotrichosis. *Journal of Investigative Dermatology*, **24**, 397.

STONE, O.J. (1971) What are the non-endocrine biologic effects of iodides? *Medical Times*, **99**, 143.

STONE, O.J. & WILLIS, C.J. (1967) Iodide enhancement of inflammation—experimental with clinical correlation. *Texas Reports on Biology and Medicine*, **25**, 205.

TACHAU, P. (1928) Erythema Exsudativum Multiforme und Nodosum. In: *Handbuch der Haut und Geschlechtskrankheiten* (Ed. by J. Jadassohn); Bd VI/2, p. 655. Springer-Verlag, Berlin.

VILANOVA, X. & AGUADÉ, J. PIÑOL (1956) Hypodermite nodulaire subaiguë migratrice. *Annales de Dermatologie et de Syphiligraphie, (Paris)*, **83**, 369.

VILANOVA, X. & AGUADÉ, J. PIÑOL (1959) Subacute nodular migratory panniculitis. *British Journal of Dermatology*, **71**, 45.

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Case Reports

Chronic mucocutaneous candidiasis treated with transfer factor

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SUMMARY

A patient with chronic mucocutaneous candidiasis resistant to all topical therapy has had extensive tests of immunological function carried out before and after administration of transfer factor. Immunological testing has been both specific, directed at responses to candida antigen, and non-specific, directed at general assessment of the patient's immune status. Transfer factor has been administered on three occasions in the past year. After each treatment temporary clinical improvement accompanied by changes in both specific and non-specific immunological responses have been observed. The possible mode of action of transfer factor in this case is discussed.

The clinical value of dialysable leukocyte extract or transfer factor (TF) in patients with defective cellular immune responses was first recorded by Lawrence over 20 years ago and since this time reports of its value in the management of viral and fungal diseases, immunodeficiency states, and malignancies have accumulated steadily (Lawrence, 1974a,b). Despite this therapeutic record the precise structure of TF and its point or points of action in the immune system have not yet been defined.

The value of TF in chronic mucocutaneous candidiasis is well recorded (Schulkind *et al.*, 1972; Kirkpatrick, Rich & Bennett, 1971; Valdimarsson *et al.*, 1972) and for this reason a patient presenting with chronic mucocutaneous candidiasis has undergone intensive immunological assessment before and after TF administration.

MATERIALS AND METHODS

The responsiveness of the patient's peripheral blood lymphocytes was assessed by incorporation of ^{14}C thymidine at 3 days in culture after stimulation with phytohaemagglutinin (PHA) at a concentration of 1/10, and pokeweed mitogen, (PWM) (Baker & Farnes) at a concentration of 1/100. The specific lymphocyte response to candida antigen was measured by the method of Valdimarsson *et al.* (1972).

Antibody to candida antigen was estimated by the radiolabelled antiglobulin technique of Nielsen, Parratt & White (1973). Leukocyte migration inhibition to candida antigen was carried out according to the method of MacKie *et al.* (1972). Intradermal skin tests were carried out to Mantoux 1/1000, Candida antigen, *T. rubrum* (Bencard), mumps (Eli Lily) and varidase (Lederle). All tests were read at 20 min and 48 h.

Dialysable TF preparation. 400 ml blood were obtained from donors who gave positive 48 h skin tests to PPD, candida and varidase. Leukocytes were isolated, washed and disrupted by freezing and thawing 9 times, followed by sonication. The extract was then filtered, lyophilized, reconstituted with sterile water and checked for sterility. It was administered by subcutaneous injection into all four limbs.

Case history

A 25-year-old Caucasian female presented with a 20-year history of oral candidiasis, a 13-year history of candidal paronychia and vaginitis, and a 3-year history of keratitis considered to be candidal in origin. There was no family history of candidiasis or immunodeficiency, but the patient had had recurrent respiratory infections throughout childhood, some of which necessitated hospital admission. In infancy she had chickenpox, measles, mumps, scarlet fever and whooping cough, all of which ran a normal course; at the age of 12 years, she had bilateral herpes zoster. One pregnancy and 3 years' oral contraceptive therapy had not affected her condition and continuous therapy with topical anti-candida agents had had no beneficial effect on her lesions.

On examination the patient had extensive oral candidiasis, paronychia involving both thumbs and the right index finger, vaginitis, and keratoconjunctivitis involving the right eye with possible early involvement of the left eye.

General examination revealed an otherwise healthy patient with no endocrinological abnormalities. *Candida albicans* was isolated on several occasions from oral, vaginal, and paronychial swabs. Serum iron was at the lower limit of normal on one occasion but on four other occasions was within normal limits. Other tests carried out with normal or negative results included full blood count, serum urea, electrolytes and calcium, liver function tests, chest X-ray and total serum iron binding capacity.

Transfer factor (16 ml) was administered subcutaneously in December 1973. Within 1 week of therapy the vaginal discharge had decreased, and within 1 month the paronychia and keratoconjunctivitis had become much less troublesome. Four months after the first dose of transfer factor her condition was static, and in view of this, and of the accompanying changes in immunological function tests, a second dose was given in May 1974. Forty-eight hours after this, she experienced a

TABLE 1. Candida specific tests of immune function

	Before TF	After TF
Lymphocyte transformation to candida	–	–
Migration inhibition factor production to candida	–	+
48 h skin tests to candida	–	+
Anti candida antibodies IgG	High	Falling
A	–	Normal
M	Low	Normal

+, positive; –, negative.

brief exacerbation of her oral and vaginal lesions, followed by complete clearing of lesions at three sites for 4 months. The candida paronychia also became quiescent for this period.

Because of clinical and immunological deterioration, further treatment with TF was carried out in November 1974. Once again clinical benefit was observed within the week of therapy. This improvement was maintained for 4 months when mild deterioration in oral and vaginal lesions were noted. It is anticipated that the patient will continue to require TF therapy at regular intervals to maintain her clinical improvement. Throughout TF therapy vigorous topical treatment with nystatin has been maintained in an effort to reduce the antigen load.

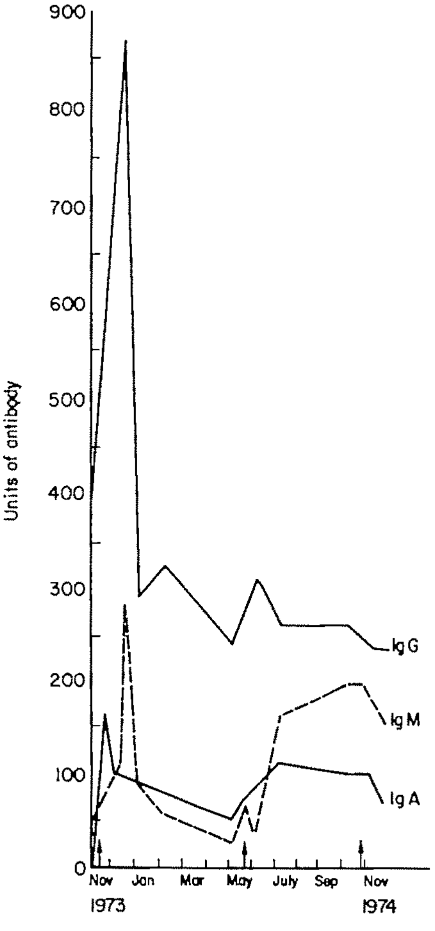

FIGURE 1. Specific anti candida IgG, A and M levels before and after TF therapy. Normal levels are 100 units for each immunoglobulin class. Arrows indicate treatment with TF.

Immunological observations

The candida-specific tests carried out and their results are outlined in Table 1. Prior to TF therapy there was no lymphocyte transformation on exposure to candida antigen, no migration inhibition factor (MIF) was observed on exposure of the patient's leukocytes to candida, and 48 h skin tests to candida were negative. Specific anti-candida antibodies were abnormal, with a high level of IgG, low IgM and absent IgA. In view of the history of repeated bacterial infections, tests of neutrophil function including the NBT tests were performed, and were within normal limits.

After TF therapy, candida mediated lymphocyte transformation remained negative, but candida induced MIF production and candida skin tests became positive; this positivity was maintained for 8–10 weeks. Reversion of these two tests on all three occasions preceded clinical deterioration. After TF administration on the first occasion the specific anti-candida IgG level rose rapidly to a very high level, and thereafter rapidly fell to below pretreatment values. Anti-candida IgM also rose temporarily, and anticandida IgA was observed for the first time. Sequential antibody changes are illustrated in Fig. 1. Total antibody levels of these three subclasses were normal before therapy, and remained unchanged during treatment.

The most significant finding in the non-specific tests of immune function prior to therapy was the low lymphocyte response to PHA and high response to pokeweed mitogen. This 'reversed ratio', suggesting an inbalance between the T and B lymphocyte systems, was corrected after the first dose of TF and has remained normal since then. The patient had a negative Mantoux test at 1/1000 despite a history of BCG vaccination 10 years previously. After the first dose of TF from a Mantoux positive donor, this reaction became positive and has remained so.

An interesting observation was the activation of the sites of skin tests within 4 h of TF administration. Seventy-two hours prior to TF administration the patient had received intradermal skin tests to Mantoux 1/1000, mumps, *T. rubrum*, candida and varidase. At 48 h, only varidase was positive, but after TF from a donor, who was skin test positive to Mantoux and candida, both those sites became markedly inflamed and indurated.

DISCUSSION

The classification of chronic mucocutaneous candidiasis is at present complex; the patient here discussed does not suffer from any of the clearly defined immunodeficiency syndromes, from familial candidiasis or from the candida-endocrinopathy syndrome. She therefore falls into the group of diffuse chronic nongranulomatous mucocutaneous candidiasis who are found to have a variety of defects of cell mediated immunity in the absence of iron deficiency. It would appear to be the first case recorded in which specific anticandida antibodies have been measured.

In this case it would appear that the patient has a basic imbalance in humoral and cell mediated responses to candida leading to overproduction of anti-candida IgG, and complete failure of cell mediated immune response to candida. TF partially restores cellular immune function as indicated by temporary MIF production and positive skin tests. The persistent lack of transformation of the patient's lymphocytes on exposure to candida shows, however, that this is not a complete correction. A possible explanation of this sequence of events is that there is in this patient a lack of a sub-population of T cells which control antibody synthesis and that TF has acted on this sub-population in stimulating cell mediated responses, and secondarily controlling humoral responses.

ACKNOWLEDGMENTS

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REFERENCES

KIRKPATRICK, C.H., RICH, R.R. & BENNETT, J.E. (1971) Chronic mucocutaneous candidiasis. Model building in cellular immunity. *Annals of Internal Medicine*, 74, 955.

LAWRENCE, H.S. (1974a) *Transfer Factor in Cellular Immunity. The Harvey Lectures series* 68, pp. 239–350. Academic Press, New York.

LAWRENCE, H.S. (1974b) Selective immunotherapy with transfer factor. In: *Advances in Clinical Immunology* (Ed. by F. H. Baer and R. A. Good), vol. 4, pp. 115–152. Academic Press, New York.

MACKIE, R.M., SPILG, W.G.S., THOMAS, C.E. & COCHRAN, A.J. (1972) Cell mediated immunity in patients with malignant melanoma. *British Journal of Dermatology*, 87, 523.

NIELSEN, K.H., PARRAT, D. & WHITE, R.G. (1973) Quantitation of antibody to particulate antigens using a radiolabelled immunoglobulin reagent. *Journal of Immunological Methods*, 3, 301.

SCHULKIND, M.L., ADLER, W.H., ALTERMIER, W.A. & AYOUB, E.M. (1972) Transfer factor in the treatment of a case of chronic mucocutaneous candidiasis. *Cellular Immunology*, 3, 606.

VALDIMARSSON, H., WOODS, C.B.S., HOLT, P.J.L. & HOBBS, J.R. (1972) The use of transfer factor in chronic mucocutaneous candidiasis. *Clinical and Experimental Immunology*, 11, 151.

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Pneumatosis cystoides intestinalis in systemic sclerosis

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SUMMARY

Pneumatosis cystoides intestinalis (PCI) is a rare condition characterized by the presence of multiple gas containing thin walled cysts in the intestinal wall and mesentery. It is sometimes associated with chronic obstructive pulmonary disease, but has been described most often in patients with gastrointestinal disorders including duodenal and gastric ulceration, small bowel obstruction, regional enteritis and gastrointestinal malignancy.

Its association in the patient described below with severe systemic sclerosis is of particular interest. There have been occasional previous reports of this association which should be considered in any patient with systemic sclerosis who develops abdominal symptoms suggestive of acute or sub-acute intestinal obstruction, not readily explicable by other causes.

CASE REPORT

Mrs I.K., aged 56 years, started with symptoms suggestive of Raynaud's phenomenon affecting the hands at the age of 24 years, for which she had a bilateral cervical sympathectomy at the age of 34. During the past 15 years she had noted increasing flexion deformity of the fingers of both hands with recurrent ulceration over metacarpophalangeal and interphalangeal joints, with the extrusion of white calcareous material. She had also noticed tightness of the skin of her face and forehead, and difficulty in fitting her dentures into the mouth. Further symptoms included a 15 years history of upper retrosternal dysphagia with regurgitation.

She had also developed small persistent ulcers over the medial malleoli and over pressure areas on the feet. She complained of arthralgia but no muscular creaking. Bowel action had been regular with light-coloured motions and occasional diarrhoea. Her appetite had remained fairly good but she had had a gradual loss of weight to 5 st 10 lb. There was no abnormality of micturition. No family history was obtainable as she had been an adopted child. On 25.6.73 she was seen at her home by one of us (L.C.B.) with a history of abdominal distension and generalized intermittent colicky abdominal pain for 2 weeks. There had been occasional vomiting at the onset and she had noticed increasing but not absolute constipation and difficulty in passing flatus. She had had no diarrhoea or passage of slime or blood per rectum. Examination showed an emaciated female with generalized pigmentation and clinical anaemia. There was no obvious dehydration. She had numerous small 'mat' telangiectases of the face and forehead, pronounced beaking of the nose and tightness of the skin of the face and forehead with a small mouth opening and angular furrowing. The fingers were held in fixed flexion and were thin and atrophic with marked beaking of the nails and loss of pulp tissue, together with small areas of calcinosis cutis over the interphalangeal and metacarpophalangeal joints.

There was generalized distension of the abdomen with visible peristalsis. There were visible superficial veins and marked tympanites on percussion. No mass was palpable and no fluid thrill or shifting dullness could be elicited. On auscultation the bowel sounds were increased whilst rectal examination showed no abnormality other than an empty rectum.

A diagnosis of systemic sclerosis with subacute intestinal obstruction was made and she was admitted to hospital for observation. A plain radiograph of the abdomen showed the presence of subphrenic gas suggesting an intestinal perforation and there was also generalized gaseous distension of both large and small bowel.

A laparotomy via a right paramedian incision revealed odourless free gas in the abdomen with a little free clear fluid. There were multiple tiny aerocysts in the wall of most of the small intestine but no definite perforation was seen nor was there any apparent obstruction.

The abdomen was closed in layers and the wound healed well. She was discharged home on 5.7.73.

She was re-admitted on 26.7.73 following a recurrence of the same symptoms which on this occasion resolved with expectant treatment. She later had further investigations of small bowel function to exclude hypocalcaemia and hypokalaemia as a cause of intestinal atony.

Investigations

Normal or negative. Serum electrolytes, phosphorus, magnesium, B 12, red cell folate, urea, aldolase. Antinuclear factor, faecal fat excretion, liver function tests, Schilling Test, creatinine clearance test. White cell count and differential.

Abnormal. Hb 11·2 g/100 ml. ESR 24 mm in 1st h. Serum calcium 8·1 mg/100 ml. Serum iron 45 μg/100 ml. Serum protein, total 6·8 g/100 ml; albumin, 3·2 g/100 ml; globulin, 3·6 g/100 ml; electrophoresis showed increased gammaglobulin. Serum immunoglobolins IgA 380, IgG more than 2000, IgM 105 mg/100 ml. D.A.T. positive 1 in 20. MSU viable count greater than 70,000/mm^3. ECG, low voltage in all waves with right axis deviation. Chest X-ray showed 'some increase in basal lung markings with no active lung disease. Multiple calcified lesions in both upper zones and a small amount of free gas beneath right dome of the diaphragm.'

She was treated with a total dose infusion of iron, intravenous Parenterovite, a single IM injection of vitamin D, and calcium gluconate and magnesium. She was also given Vivanex (an elemental diet) daily in view of her very poor nutritional state. Since discharge from hospital she has had no further abdominal symptoms and at her last review on 29.8.74 there was only slight distension of the abdomen with some increased resonance on percussion.

DISCUSSION

Pneumatosis cystoides intestinalis (PCI) was first described by Du Vernoi in 1730 and later in England by John Hunter in the intestines of a hog. It is an uncommon condition characterized by the presence of numerous gas containing 'cysts' in the intestinal wall. The small intestine and colon particularly in the caecal region are the areas usually involved.

Gas cysts may also be present in the mesentery and retroperitoneal tissues. In an extensive review of 213 cases, Koss (1952) found the small intestine to be affected in 63% and the colon in 22%.

The cysts vary in size from a pin head to several cm and contain variable mixtures of gases on analysis. Usually the oxygen tension is decreased and there is an increased concentration of carbon dioxide up to 15%. Hydrogen is sometimes found, presumably produced by intraluminal bacterial activity. Spontaneous rupture of cysts gives rise to pneumoperitoneum which may become recurrent or chronic.

PCI is frequently associated with obstructive pulmonary diseases but has been described most often in patients with gastrointestinal disorders, including gastric and duodenal ulcer, small bowel obstruction, regional enteritis and malignant disease of the intestinal tract. In the past, few sporadic reports have noted an association with systemic sclerosis (Meihoff, Hirschfield & Kern, 1968; Gompels, 1969; Miercourt & Merrill, 1969). All these patients including the patient described in this report had obvious systemic sclerosis but only two of three cases reported by Meihoff *et al.* (1968) had obvious skin changes at the onset of the intestinal symptoms.

A dietary deficient state has been postulated as a cause of the condition but so far no sound experimental evidence has supported this theory. Naeslund (1924) suggested that gas-forming organisms in the gut might be the cause of PCI, as by analogy a similar condition occurs in swine, although in man, pneumoperitoneum secondary to PCI giving rise to peritonitis is virtually unknown.

Mechanical pressure may cause gas to pass from the lumen of the bowel into the lymphatics and could account for the association with peptic ulcer, especially if accompanied by pyloric stenosis. Here gas may be formed through an ulcerated area and track interstitially within the bowel wall. Keating *et al.* (1961) have shown that in animals air can track from the mediastinum to the root of the mesentery and suggested that rupture of pulmonary alveoli in patients with bronchial asthma and emphysema may produce PCI by this route.

The radiological diagnosis of PCI may be difficult since the recognition of abdominal gas shadows within the intestinal wall from normal gas in the lumen may not be possible. Occasionally the gas is seen in the form of a linear stripe or a double walled ring-like shadow which is seen when the lumen is viewed end on. Gas streaks may also be seen in the retroperitoneal tissues and rarely in the mediastinum. Rupture of the intestinal cysts gives rise to pneumoperitoneum which is invariably sterile. The clinical picture is usually overshadowed by the presence of pyloric obstruction and the existence of cysts first revealed at operation, although a plain X-ray of the abdomen may show some of the above changes.

Indecisive abdominal symptoms may be a feature, including vague generalized colicky abdominal pain, diarrhoea with mucus and excessive flatus, some of the symptoms being suggestive of a malabsorption state and indeed malabsorption secondary to pneumatosis of the small bowel has been described by Yunich & Fradkin (1958). In systemic sclerosis there is involvement of both the respiratory system and the gastrointestinal tract. In the duodenum, jejunum, ileum and colon there is a patchy disappearance of muscle especially in the circular layer with replacement by fibrous tissue. The subserosa and submucosal tissue may also be thickened and fibrosed, the vessels in these areas showing sclerosis and intimal fibrosis. Likewise in the lungs there may be progressive diffuse alveolar fibrosis with obliteration of capillaries and alveolar spaces. Rupture of thickened alveolar walls may give rise to thin-walled gas containing cysts. It is possible therefore that PCI in systemic sclerosis may have a similar causation to PCI described with respiratory disorders, with rupture of distended pulmonary alveolar cysts, the gas tracking through the diaphragm and ultimately to the mesentery.

It is, however, more likely that the gas cysts are formed locally in the intestinal wall consequent upon the local ischaemic changes, the cysts then rupturing to give rise to pneumoperitoneum. Rowell (1972) supports this theory of causation.

In systemic sclerosis there is also a chronic partial intestinal obstruction associated with changes outlined above, although the mucous membrane itself often appears normal on histology. This obstruction allows massive bacterial contamination to occur in the proximal part of the small bowel producing a 'blind loop' syndrome leading at times to malabsorption.

Extensive investigation in this patient in spite of gross wasting did not show any evidence of biochemical malabsorption except perhaps for the low serum calcium and iron.

It has been suggested (Meihoff *et al.*, 1968) that the occurrence of PCI in systemic sclerosis is of ominous prognostic significance in contrast to PCI occurring with other conditions. White, Treece & Juniper (1970) suggested that this gloomy prognosis is not invariable and described one patient who survived 6½ years. Our patient has had two episodes of PCI and has had no recurrence of symptoms up to July 1975. Recently Forgacs, Wright & Wyatt (1973) suggested that by altering the balance between diffusion of gases in and out of cysts, by lowering the total pressure of gases in the venous blood, it should be possible to 'degas' the cysts. This they achieved by allowing the patient to breath oxygen in a 75% gas mixture with nitrogen; as the administration had to be continuous, a heat tent was used continuously for 6 days and was apparently well tolerated. Cotterill (1974) has used this form of treatment with good results in a patient with pulmonary emphysema and a large solitary aerocyst of the iliac colon.

From the case reported above and from other similar sporadic cases reported in the literature it is suggested that this condition should be considered in any patient with systemic sclerosis who develops symptoms of acute or subacute intestinal obstruction not readily explicable by other causes. If the diagnosis can be confirmed with a plain X-ray of the abdomen it may be possible to avoid laparotomy, the condition being treated on conservative lines.

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REFERENCES

Cotterill, J.A. (1974) Personal communication.

Du Vernoi, J.G. (1730) *Observations of the Anatomical Academy of Science Peropol*, 5, 213.

Forgacs, P., Wright, P.H. & Wyatt, A.P. (1973) Treatment of intestinal gas cysts by oxygen breathing. *Lancet*, **i**, 579.

Gompels, B.M. (1969) Pneumatosis cystoides intestinalis associated with progressive systemic sclerosis. *British Journal of Radiology*, **42**, 701.

Keating, W.S., McCarver, R.R., Kovarik, J.K. & Daywith, A. (1961) Pneumatosis intestinalis, a new concept. *Radiology*, **76**, 733.

Koss, L.G. (1952) Abdominal gas cysts: analysis with report of a case and critical review of literature. *Archives of Pathology*, **53**, 523.

Meihoff, W.E., Hirschfield, J.S. & Kern, F. (1968) Small intestinal scleroderma with malabsorption and pneumatic cystoides intestinalis. A report of three cases. *Journal of American Medical Association*, **204**, 102.

Meircort, R.D. & Merrill, F.G. (1969) Pneumatosis and pseudo-obstruction in scleroderma. *Radiology*, **92**, 359.

Naeslund, J. (1924) Zur Kenntnis der Pneumatosis Cystoides Intestinalis. *M.D. Thesis*. Uppsalla Mareus, Sweden.

Rowell, N.R. (1972) Lupus erythematosus, scleroderma and dermatomyositis. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J. Ebling), Vol. 2, p. 1112. Blackwell Scientific Publications, Oxford.

White, W.D., Treece, T.R. & Juniper, K. (1970) In letter to Editor (pneumatosis in scleroderma of small bowel). *Journal of American Medical Association*, **212**, 1068.

Yunich, A.M. & Fradkin, N.F. (1958) Malabsorption syndrome secondary to extensive pneumatosis cystoides intestinalis. *Gastroenterology*, **35**, 212.

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Postoperative progressive gangrene: a reminder

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SUMMARY

A classical example of progressive postoperative gangrene is described and the relevant literature has been reviewed.

After consideration of the evolution and the clinical appearances of the condition, of the consistency of the associated bacterial flora and of the response to antibiotic therapy alone, we suggest that progressive postoperative gangrene is a distinct entity and separate from pyoderma gangrenosum.

Progressive postoperative gangrene is a slowly spreading superficial painful ulcer which develops following operation, usually for a suppurative abdominal or chest condition. A microaerophilic streptococcus is aetiologically implicated. Untreated the ulceration enlarges inexorably and death from inanition is the likely outcome.

The first case of the condition was probably described by Luckett (1909) though Cullen (1924) and Christopher (1924) are usually credited with first recording the condition as a distinct entity. It has variously been called 'progressive enlarging ulcers of the abdominal wall' (Cullen, 1924), 'severe spreading carbunculus infection of the chest wall' (Christopher, 1924), 'progressive gangrene infection of the skin and subcutaneous tissue' (Brewer & Meleney, 1926), 'progressive gangrenous ulceration of the abdominal wall' (Shipley, 1928), 'postoperative progressive gangrenous infection of skin and subcutaneous tissue' (Probstein & Seelig, 1928), 'postoperative spreading superficial gangrene' (Alexander, 1926) and 'postoperative progressive bacterial synergistic gangrene' (Meleney, 1933).

The term postoperative progressive gangrene (PPG) (Ballin & Morse, 1931) is preferred here as it embraces three of the salient unequivocal features of the disease. The total number of reported cases is small, being in the region of 100 cases. Reports in the dermatological literature are particularly rare and, additionally, are sceptical of the existence of the condition as an entity (Greenbaum, 1942; Güller, 1968).

It is our intention with this report to remind dermatologists of PPG. A classical case which was successfully treated by systemic antibiotics is reported; the salient features of the disease, in terms of its bacteriology, clinical features, differential diagnosis and treatment are presented as they emerge

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from a review of the literature; and arguments in favour of regarding PPG as a specific entity are presented.

CASE REPORT

Miss G.B. a 27-year-old cup maker who had previously enjoyed good health, was admitted to the North Staffordshire Royal Infirmary, Stoke-on-Trent, on 13 May 1972, suffering from acute appendicitis, the classical symptoms and signs having evolved over the preceding 6 h.

At operation, the same evening, a severely inflamed appendix was removed; no perforation of the organ had occurred but a small quantity of non-purulent peritoneal exudate was present. Wound closure was effected without drainage. Ampicillin 500 mg and Keflex (cephalexin—Lilly) 500 mg 6-hourly were started orally.

Post-operative progress was initially uneventful. On the 8th day after the operation, a purulent discharge from the abdominal wound was noted, associated with pyrexia (38·0°C) and tachycardia (pulse rate 140/min); the patient felt unwell and was restless. The wound edges were described by the surgical observers as 'bruised'. Swabs for bacteriological examinations were taken from the purulent material but subsequently no pathogenic organisms were cultured. Crystamycin (crystalline penicillin 500,000 units and streptomycin sulphate 500 mg) intramuscularly was introduced to replace the ampicillin and Keflex. Full blood examination at this stage revealed a total white blood count of 32,700/mm^3 (88% polymorphonuclear leukocytes); blood cultures were negative.

Over the next 5 days the patient remained pyrexial (38·5–39·5°C) without any noticeable deterioration in her general condition despite the addition of chloramphenicol 500 mg 6-hourly orally to her antibiotic therapy. During this period the skin overlying the whole of the right iliac fossa, the hypogastrium and part of the left iliac fossa had been progressively converted into a superficial gangrenous state centred on the operation wound, with separation of the necrotic skin in some areas to reveal red granulation tissue, and a carbuncular appearance at the spreading edges (Fig. 1).

On the 20th day after operation, the results of further bacteriological examinations became available. Cultures revealed a *microaerophilic streptococcus* sensitive to penicillin, ampicillin, cloxacillin, tetracycline, erythromycin, chloramphenicol, gentamicin, kannamycin, cephalosporin and trimethoprim sulphate and resistant to novobiocin; and a *coagulase negative staphylococcus* sensitive to tetracycline, erythromycin, novobiocin, chloramphenicol, gentamicin, kannamycin, trimethoprim and cephalosporin but resistant to all penicillins.

On receipt of this information, a regime of gentamicin (as Genticin Injectable) 80 mg 8-hourly, Septrin (Wellcome) two tablets 6-hourly and Fucidin (Leo) 500 mg 6-hourly was introduced, together with irrigation of the necrotic areas using 0·5% chloramphenicol in saline.

Subsequent progress (2 June–1 July, 1972). From this point onwards, the patient's clinical condition improved dramatically, the oedema of the 'wound' edges diminished and over the next 4 weeks epithelialization of the necrotic areas occurred (Fig. 2). She was discharged from hospital on 1 July 1972.

Follow-up. Over the next year she was seen at regular intervals in the Surgical Outpatients Clinic; complete healing of the skin defect was noted by 4 August 1972.

In September 1973, the patient attended the Skin Department for advice concerning warts on the hands when an 'incidental finding' was the scarring on the lower abdomen (Fig. 3).

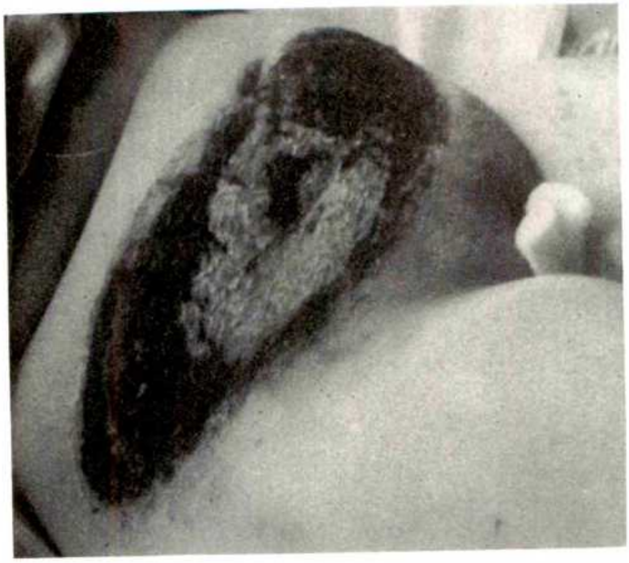

FIGURE 1. The ulcer on the 20th postoperative day. Peripherally there is a wide zone of gangrene with a central slough. This has separated on the left, to reveal granulation tissue.

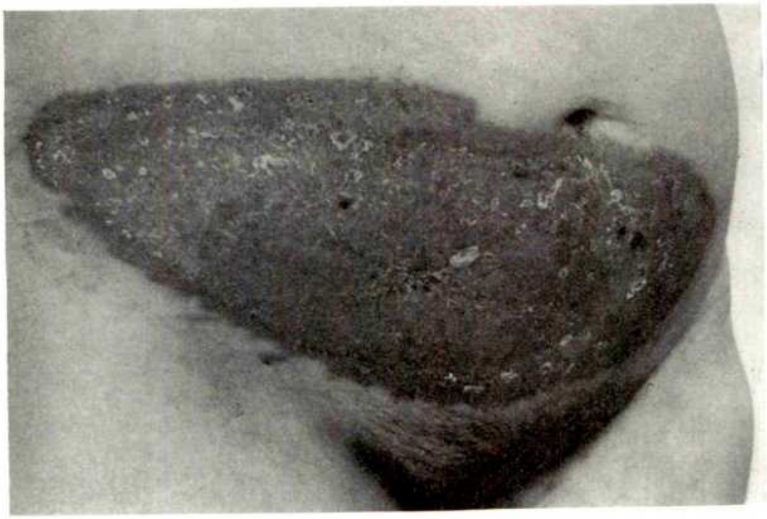

FIGURE 2. The healed lesion 7 weeks after operation. The ulcer has re-epithelialized.

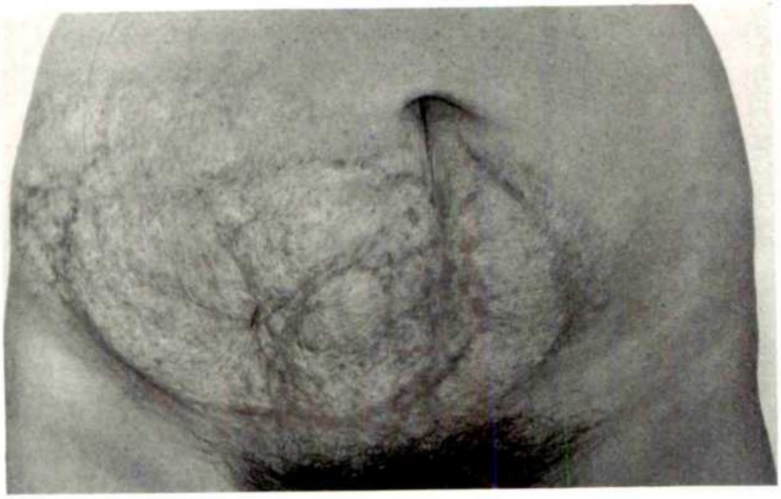

FIGURE 3. The healed ulcer 14 months following the operation.

DISCUSSION

The significant features of this case are, firstly, the onset of the ulceration following abdominal operation, secondly, the isolation of an anaerobic streptococcus, and thirdly, the successful treatment of the condition with systemic antibiotics.

From a review of the literature the following clinical picture of the disease emerges.

Incidence. The disease is rare and even before the introduction of antibiotics it was estimated that a surgeon would see only one case in a working lifetime (Vier, 1940). This estimate was supported by our senior surgical colleagues.

Predisposing factors. The all-important predisposing factor is a recent abdominal or thoracic operation. The types of operation which have been reported to have been associated with PPG are listed (Table 1). It is apparent that operations involving the drainage of pus are particularly frequent. Any age group may be affected and there is a male predominance of about 4:1. The general health of the patients prior to the onset of the disease is usually good.

Aetiology. Meleney was the first to demonstrate a consistent bacteriological pattern which comprised two elements.

A microaerophilic non-haemolytic streptococcus was isolated from the periphery of the ulcer, and even from the excised adjacent normal tissue. The streptococcus was described as *Streptococcus evolutus* of Prevot. The same organism was subsequently isolated from other lesions (Patterson, 1932; Willard, 1936; Lyall & Stuart, 1948; de Jong, Smith & Thoma, 1967). *Staphylococcus aureus* and a variable number of Gram negative rods, principally Proteus, were isolated from the central necrotic tissue. Meleney successfully produced gangrenous lesions in animals by the simultaneous injection of the micraerophilic streptococcus and *Staphylococcus aureus*, which could not be produced by the injection of either organism individually. Willard (1936) repeated this experiment and Lyall & Stuart (1948) demonstrated that Proteus and *Staphylococcus albus* could also play the synergistic role with the

TABLE I. Surgical conditions reported to have been complicated by postoperative progressive gangrene*

Site	Condition	Relative frequency (%)
Abdomen/pelvis	Appendix abscess	49
	Subphrenic abscess	7
	Perforated peptic ulcer	4
	Purulent cholecystitis	2
	Simple appendicitis	13
	Chronic cholelithiasis	2
	Carcinoma of caecum	2
	Ventral hernia	2
	Oophorectomy	2
	Adenocarcinoma of endometrium and pyometra	2
Chest	Empyema	13
Scrotum	Epididimyitis	2
Breast	Abscess	2

* From forty-five cases reported in the literature.

microaerophilic streptococcus to produce gangrenous lesions experimentally. The latter authors emphasized that the microaerophilic streptococcus was the critical factor.

Clinical features. The onset of the disease is usually 7–14 days following the operation, extremes being 1 day (Meleney, 1931) to 7 weeks (Grimshaw & Stent, 1945). The wound becomes acutely inflamed and the suture holes develop a carbuncular appearance. The centre of activity becomes purplish in colour while the outer zone is bright red and the wound is characteristically extremely tender. After 2–3 weeks the central skin undergoes necrosis and later separates off to leave red granulation tissue. A narrow peripheral necrotic rim may remain which merges with the raised serpiginous edge. Outside this zone there is an erythematous rim which may be several centimetres wide. According to Meleney there is little undermining of the edge of the ulcer in PPG, in contradistinction to the situation in the 'burrowing ulcer' (Pyoderma Gangrenosum). Other authors describe the ulcer edge in PPG as being undermined (Ballin & Morse, 1931; Gillespie, 1928; Touraine & Duperrat, 1939). In our case we could detect no clinical distinguishing features of the ulcer morphology from those of pyoderma gangrenosum. There follows a slow inexorable spread of the ulcer, at a rate of approximately 4 mm per day (Alexander, 1926), so that the whole abdomen and beyond may be involved. Descriptions of horrific ulcers extending over most of the abdomen and back, from the neck to the sacrum, are frequent (Christopher, 1924; Poate, 1930; Stuart-Wallace, 1935; Lyall & Stewart, 1948). Despite its lateral extent the ulcer remains relatively superficial and does not penetrate beneath the deep fascia.

Systemic reaction in the initial period is minimal, being limited to a low grade fever. With time and spread of the lesion, however, there is increasing inanition and death is the usual outcome if the condition is untreated.

Differential diagnosis. The principal differential diagnoses are the other infective gangrenes and pyoderma gangrenosum.

Acute streptococcal gangrene is usually situated on the limbs and produces much local inflammation and swelling which progresses to gangrene in a few days. There are profound systemic effects and usually the condition has a fulminating course. Haemolytic streptococci can be isolated from the lesion and from blood culture. Gas gangrene is similarly associated with severe local inflammation, rapid progression and prostration. Additionally gas is present in the tissues and Clostridia are easily isolated from the wound. Fusospirochaetal gangrene follows human bites or the contamination of wounds with mouth secretions. The ulcer develops in a few days and has shaggy bleeding edges and produces foul smelling pus. There is a tendency to deep burrowing with the formation of multiple sinus tracts. Amoebic ulcers of the skin are usually associated with the drainage of deep abscesses. The ulcer enlarges more rapidly than in PPG. The granulations of the ulcer base are described as resembling the appearance of raw beef and amoebae are present in the wound and stool.

The ulcer of pyoderma gangrenosum may closely resemble PPG. Points of differentiation are the absence of gangrene, the absence of a recent operation and the absence of specific bacteriological findings in pyoderma gangrenosum. However, distinction between the two entities may not be simple. The gangrenous zone in the developed ulcer of PPG may be very narrow and inconspicuous. It is also conceivable that pyoderma gangrenosum could occur in a postoperative situation as trauma is a known precipitating factor of pyoderma gangrenosum.

Diagnosis. We feel that the important diagnostic points in PPG are the history of recent abdominal or thoracic surgery, usually for a suppurative condition, the superficiality of the usually solitary ulcer, the slow spread of the ulcer with lack of early significant toxic systemic effects, and finally the isolation of a microaerophilic streptococcus from the ulcer edge.

Treatment. Before the antibiotic era, cases were successfully treated by surgery, either by radical excision or by cautery. Local antiseptics were singularly ineffective. However, the therapeutic ingenuity of Coakley & Klein (1936), who apparently employed maggots, was rewarded with success! More recent cases have been cured with antibiotics, penicillin (Meleney *et al.*, 1945; Grimshaw & Stent, 1945), bacitracin (Meleney, Shambaough & Millen, 1950) and now in our case with the combination of gentamicin, Fucidin and Septrin.

As mentioned previously, there has been scepticism in the dermatological literature regarding the individuality of PPG. The most serious challenge was from Güller (1968) who reported a case of PPG which was cured by steroids, having failed to respond to systemic antibiotics. His conclusion was that PPG was in reality pyoderma gangrenosum. There are several points in the report, however, which raise the suspicion that the label of PPG should not have been used in this case. The lesions occurred on the legs while most reported cases occurred on the abdomen or chest and in circumstances where the relevant organism might have infected the skin from an abdominal or pulmonary source. Also in Güller's case there were three ulcers, while most of the previously reported ulcers were solitary. Lastly the bacteriological cultures were sterile.

Similarly, confusion may arise from the surgical literature because of an over-embracing definition of PPG. Meleney for example, who probably had the most extensive experience of the condition, may well have included cases of pyoderma gangrenosum in his case reports of PPG. In two of five cases described as PPG (Meleney *et al.*, 1950), there were multiple leg ulcers in patients known to have ulcerative colitis.

We feel that there are sufficient reasons for considering PPG as a distinct entity: firstly, there is the consistent demonstration of organisms, in particular the microaerophilic streptococcus where appropriate cultural methods have been used; secondly, the fact that lesions are restricted to situations where infection by this organism is possible; thirdly, the characteristic inevitable untreated course and the dramatic response to the appropriate systemically administered antibiotics.

REFERENCES

Alexander, E.G. (1926) Postoperative spreading-superficial gangrene. *Annals of Surgery*, **34**, 461.

Ballin, M. & Morse, P.F. (1931) Progressive postoperative gangrene of the skin. *American Journal of Surgery*, **11**, 81.

Brewer, G.E. & Meleney, F.L. (1926) Progressive gangrenous infection of the skin and subcutaneous tissue following operation for acute perforative appendicitis: a study in symbiosis. *Annals of Surgery*, **34**, 438.

Christopher, F. (1924) Severe spreading carbuncular infection of chest wall following rib resection under local anaesthesia. *Surgical Clinics of North America*, **4**, 795.

Coakley, W. & Klein, S. (1936) Progressive postoperative gangrene of skin. Report of case. *American Journal of Surgery*, **33**, 287.

Cullen, T.S. (1924) A progressively enlarging ulcer of the abdominal wall involving the skin and fat, following drainage of an abdominal abscess apparently of appendiceal origin. *Surgery, Gynecology and Obstetrics*, **38**, 579.

Gillespie, M.G. (1928) Phagedaenic ulceration of the skin. *Annals of Surgery*, **88**, 248.

Greenbaum, S.S. (1942) Phagedaena geometrica (Brocq). *Archives of Dermatology and Syphilology*, **43**, 775.

Grimshaw, C.K. & Stent, L. (1945) Postoperative cutaneous gangrene: effect of penicillin. *Lancet*, **248**, 434.

Güller, R. (1968) Postoperative progressive Gangrän und Dermatitis ulcerosa. *Hautarzt*, **19**, 408.

de Jong, D.S., Smith, J.P. & Thoma, G.W. (1967) Postoperative synergistic gangrene. *Journal of the American Medical Association*, **200**, 227.

Luckett, W.H. (1909) Large phagedenic ulcer of the abdomen. *Annals of Surgery*, **50**, 605.

Lyall, A. & Stuart, R.D. (1948) Progressive postoperative gangrene of the skin: observations on aetiology and treatment in two cases. *Glasgow Medical Journal*, **29**, 1.

Meleney, F.L. (1931) Synergism in disease processes. *Annals of Surgery*, **94**, 961.

Meleney, F.L. (1933) A differential diagnosis between certain types of infectious gangrene of the skin: with a particular reference to haemolytic streptococcus gangrene and bacterial synergistic gangrene. *Surgery, Gynecology and Obstetrics*, **56**, 847.

Meleney, F.L., Friedman, S.T. & Harvey, H.D. (1945) The treatment of progressive synergistic gangrene with penicillin. *Surgery*, **18**, 423.

Meleney, F.L., Shambaough, P. & Millen, R.S. (1950) Systemic bacitracin in the treatment of progressive bacterial synergistic gangrene. *Annals of Surgery*, **131**, 129.

Patterson, H.A. (1932) Progressive postoperative cutaneous gangrene. *Annals of Surgery*, **96**, 1091.

Poate, R.G. (1930) Spreading gangrenous inflammation. *Medical Journal of Australia*, **2**, 398.

Probstein, L.G. & Seelig, M.G. (1928) The treatment of the postoperative progressive infection of the skin and subcutaneous tissue with blood from immunized donors. *Surgery, Gynecology and Obstetrics*, **47**, 247.

Shipley, A.N. (1928) Progressive gangrenous ulceration of the abdominal wall. *Annals of Surgery*, **87**, 245.

Stewart-Wallace, A.M. (1935) Progressive postoperative gangrene of the skin. *British Journal of Surgery*, **22**, 642.

Touraine A. & Duperrat, R. (1939) La gangrène post opératoire progressive de la peau. *Annaes de Dermatologie et de Syphiligraphie*, **10**, 257.

Vier, H.J. (1940) Progressive postoperative gangrene of the abdominal wall: with a case report. *Surgery*, **7**, 334.

Willard, H.G. (1936) Chronic progressive gangrene of the abdominal wall. *Annals of Surgery*, **104**, 227.

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Skin necrosis following warfarin therapy

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SUMMARY

A typical case of skin necrosis following anticoagulant therapy with warfarin is described.

Skin necrosis following coumarin anticoagulant therapy is a rare complication. Reports from Europe and the United States show that it is seen in those countries. The literature contains no British reference. We wish to record this hazard following the use of warfarin sodium.

The patient, a 52-year-old woman, in March 1974 had been prescribed warfarin for 7 weeks following thrombophlebitis in the left leg. In July 1974 she was readmitted with a diagnosis of veno-occlusive disease and pulmonary emboli. Anticoagulant therapy was started with heparin and warfarin sodium.

Her course during her second admission was as follows:

Day 1. Heparin 20,000 units 12 hourly was given intravenously and warfarin sodium 40 mg orally.

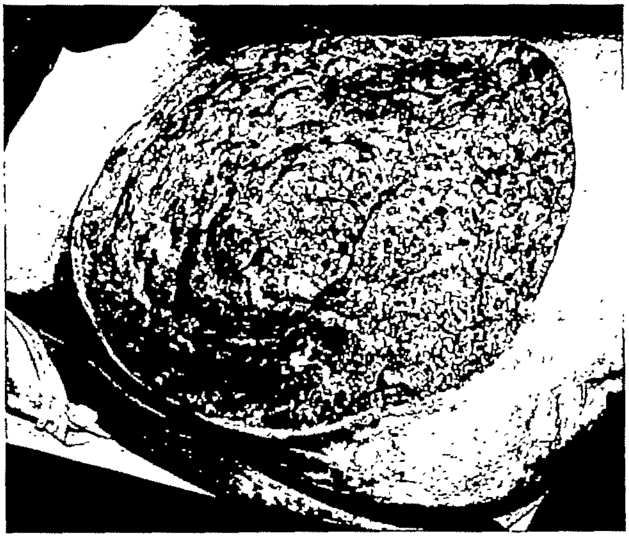

FIGURE 1. Full extent of skin and subcutaneous tissue loss from the right buttock after debridement.

Fifteen minutes after receiving warfarin she vomited and a repeat dose of 40 mg was given.

Day 4. Her prothrombin time was 44 s. She complained of painful hips, and discoloration of the skin resembling early bruising was noticed.

Day 5. The skin over both hips had developed a livid purple discoloration. The prothrombin time was 55 s (control 15 s). 20 mg vitamin K was given intravenously.

Day 6. Haemorrhagic blisters appeared over the discoloured skin on both thighs, and they became more extensive on her body during the next 24 h. Erythema and induration had appeared surrounding the infarcted skin. There was no evidence elsewhere of purpura or ecchymoses. In the next 10 days the area of necrosis formed an eschar and began to separate from the subcutaneous tissue. The area became purulent; the patient developed a pyrexia and *Staph. aureus* was isolated from the right thigh.

The necrotic skin was removed from the right hip following increasing pain and a continuing pyrexia. Extensive necrosis of fat with abscess formation was noted by the surgeon, who the next day removed all necrotic debris from the wound.

Two attempts were made to graft areas of the large wound with split skin from the left thigh. On both occasions the graft sloughed.

The wound was treated daily by simple toilet and has re-epithelialized after 4 months.

COMMENT

This patient developed extensive skin necrosis 4 days after receiving warfarin sodium on the second occasion for veno-occlusive disease. The area of tissue loss was large and extended deep into subcutaneous fat. Bacterial infection has been difficult to eradicate, and pain to control. Nursing has been a cumbersome task.

Since Verhagen & Wiersema (1952) and Verhagen (1954) reviewed this rare complication following coumarin anticoagulant therapy, further reports have followed in the European and American literature. There are more than 150 cases reported (Everitt & Overholt, 1969), but there are no references to it in the British literature. No report has been made to the Committee on the Safety of Medicines (personal communication). The majority of cases have occurred in obese middle-aged women, the lesions having a predilection for fatty areas such as thighs, buttocks, abdomen and breasts. An evanescent erythema coinciding with pain in the area affected is the first indication of the condition. In 90% of patients the complication is seen between the 3rd and 6th day of treatment. Our patient's course, progressing from erythema to purple ecchymotic changes, blistering and necrosis is typical of cases so far reported.

The indications for anticoagulant therapy in the patients reviewed by Verhagen (1954) and the cases described by Nalbandian *et al.* (1965) reveal no common underlying pathology. Reports on the incidence of cutaneous necrosis amongst the various coumarins suggest it is less common with warfarin than with bishydroxycoumarin (Dicoumarol) and other coumarins (Nalbandian *et al.*, 1965). This may reflect the varying usage of different anticoagulants in America and Europe. In 1973 289,000 G.P. prescriptions for warfarin sodium were issued in the U.K. It is now the market leader amongst anticoagulants. Sales of warfarin in the U.S.A. are approximately double our own, and in France and Germany are approximately half those in the U.K.

Noteworthy in this case and also in one reported by Nalbandian (1964) is the occurrence with the use of a coumarin for the second time. Although in most reported cases the drug has been stopped, its continuation is said not to exacerbate the condition or delay skin healing (Nalbandian *et al.*, 1965; Verhagen, 1954; Hague, 1957; Epiney, 1963). Examples have been reported with repeated use of the same coumarin failing to cause necrosis a second time and variable response in the same patient to different coumarins (Nalbandian *et al.*, 1965). Scarification and intracutaneous skin challenge produce no reaction (Stafanelli *et al.*, 1960). Such evidence would seem to preclude a hypersensitivity reaction. No histology is available from our patient but reports in the literature suggest selective involvement of skin capillaries and venules with thrombi; later haemorrhage and finally fat necrosis occur (Flood *et al.*, 1943; Sailer & Wayner, 1960; Epiney, 1963). Endothelial proliferation and hyaline necrosis of the walls of small vessels occurred as a generalized change in dogs given toxic doses of dicoumarol (McCarter, Bingham & Meyer, 1944).

The site of the initial lesions is purported to be the junction of the dermovascular capillary loop with the precapillary arteriole (Nalbandian *et al.*, 1965). The evolution of clinical changes would fit this theory, and skin petechiae arise from this site (Humble, 1949).

There is no definitive treatment described. The onset of the complication is not related to prolongation of the prothrombin time or depression of clotting factors. Early administration of heparin might be expected to inhibit thrombosis, and Beller (1961) claimed benefit with this treatment, although others have been unsuccessful with its use.

REFERENCES

BELLER, F.K. (1961) Therapy of so-called Coumarin necrosis. Abstract. *Medicine et Biologie* (*Paris*), **4**, 116.

EPINEY, J. (1963) Nécrose cutanée et anticoagulants. *Revue Médicale de la Suisse Romande*, **83**, 1011.

EVERETT, E.D., OVERHOLT, E.L. (1969) Haemorrhagic skin infarction secondary to oral anticoagulants. *Archives of Dermatology*, **100**, 588.

FLOOD, E.P., REDISH, M.H., BOCIEK, S.J. & SHAPIRO, S. (1943) Thrombophlebitis migrans disseminata: report of a case in which gangrene of the breast occurred. *New York State Journal of Medicine*, **43**, 1121.

HAGUE, J. (1957) Haemorrhagisk Hudnekrose ved Dicumarolbehandlung. *Nordiskt Medicinskt Arkiv*, **58**, 1266.

HUMBLE, J.G. (1949) Mechanism of petechial haemorrhage formation. *Blood*, **4**, 69.

MCCARTER, J.C., BINGHAM, J.B. & MEYER, O.O. (1944) Studies on haemorrhagic agent 3.3′ Methylenebis (4 Hydroxycoumarin). *American Journal of Pathology*, **20**, 651.

NALBANDIAN, R.M., MADER, I.J., BARRETT, J.L., PEARCE, J.F. & RUPP, E.C. (1965) Petechiae, ecchymoses and necrosis of skin induced by coumarin congeners. *Journal of the American Medical Association*, **192**, 107.

SAILER, S. & WAGNER, K. (1960) Thrombosen unter der Behandlung mit Antikoagulantien. *Wiener Klinische Wochenschrift*, **72**, 152.

STAFANELLI, N., TULZER, H. & WEWALKA, F. (1960) Hautnekrosen als Nebenwirkung der Therapie mit Antikoagulantien. *Thrombosis et Diathesis Haemorrhagica*, **5**, 136.

VERHAGEN, H. (1954) Local haemorrhage and necrosis of skin and underlying tissues during anticoagulant therapy with Dicoumarol or Dicumacyl. *Acta Medica Scandinavica*, **148**, 453.

VERHAGEN, H. & WIERSEMA, M.U. (1952) Huidbloeding en Necrose bij Patiënten met Thrombose behandeld met Dicumol af Dicumacyl. *Nederlandsch tijdshrift voor geneeskunde*, **96**, 1578.

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Contact urticaria and anaphylactoid reaction induced by topical application of nitrogen mustard

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SUMMARY

A 36-year-old woman and a 16-year-old boy, both suffering from mycosis fungoides, developed urticaria and an anaphylactoid reaction after topical whole body application of nitrogen mustard. Prick tests with nitrogen mustard solution produced a weal and flare response.

Both patients had previously been treated intermittently with total body applications of nitrogen mustard for 2½ years and 1 year respectively without complications.

It is well known that topically administered drugs may elicit reactions of the delayed hypersensitivity type, allergic contact dermatitis, but immunological reactions of immediate type hypersensitivity after topically applied medications are less well known (Warin & Champion, 1974). However, anaphylactic reactions have been reported following topical treatment with neomycin (Pippen, 1966), bacitracin (Comaish & Cunliffe, 1967), and an ointment containing neomycin and bacitracin (Roupe & Strannegaard, 1969) and after its application on an endotracheal catheter (Eriksen, 1963). Maucher (1972) described two patients with severe hypersensitivity reactions to systemic penicillin who developed urticaria at the penicillin patch test site that was later followed by systemic allergic reactions. Daughter, Zackheim & Maibach (1973) have reported an urticarial response and anaphylactoid reaction after topical application of mechlorethamine.

This paper describes two cases of immediate hypersensitivity reactions after topical application of nitrogen mustard, Erasol.

CASE REPORTS

Case 1

A 36-year-old woman with mycosis fungoides of 5 years' duration, in stage II according to van Scott & Kalmanson's (1973) classification, was treated intermittently with whole body applications of nitrogen mustard (40 mg of the drug in 40 ml aqueous solution per treatment). The treatment was repeated once a month, 25 times in all. After the 24th and 25th application, within 30 min she developed generalized pruritus and nausea followed by sweating, burning and a prickling sensation together with tightness of the chest. One and a half hours after treatment she had generalized urticaria.

Her pulse rate was regular with a frequency of 112 beats per min, blood pressure 130/80 mmHg. Her body temperature was 38·9°C. Auscultation of her chest was normal. Following oral antihistamine treatment (cyproheptadine 20 mg) all symptoms disappeared.

Case 2

A 16-year-old boy, with mycosis fungoides in stage II of 1½ years' duration, had been treated intermittently (once a month) with universal topical applications of nitrogen mustard, after initial 'tolerance induction' to the drug (van Scott & Kalmanson, 1973). Attempts to induce tolerance were performed by intravenous injections of 200 μg of nitrogen mustard weekly for 5 weeks.

Two days after treatment 17 and 4 months after the last intravenous injection, he developed urticarial lesions for the first time. They disappeared 5–10 min after administration of cyproheptadine. Generalized urticaria also appeared after three later topical treatments. Urticaria was accompanied by a prickling sensation, sweating and universal itching. All symptoms vanished within 1 h.

In both patients prick tests were performed with freshly prepared aqueous solutions of nitrogen mustard (Erasol) in the following concentrations: 10^{-10}, $^{-6}$, $^{-5}$, $^{-4}$, $^{-3}$, $^{-2}$. All necessary resuscitation equipment was readily available. Following nitrogen mustard at the concentration of 10^{-2} both patients produced after about 15 min an erythematous reaction which approximately 1½ h later turned into a weal and flare response limited to the test area. Five volunteers showed no reactions to nitrogen mustard at this concentration.

Serum levels of immunoglobulin E (IgE) were 76 and 62 μg/l, respectively (normal values: 60–1000 μg/l). Values of complement factors C_3 and C_4 as well as C′1-esterase inhibitor before and after topical treatment were all normal.

A biopsy from the urticarial lesions confirmed the clinical diagnosis.

DISCUSSION

Allergic contact dermatitis is a common complication of topical treatment. However, topical administration of drugs may also result in immediate type reactions.

Anaphylactoid reactions after topical treatment may occur after a patient has developed delayed hypersensitivity to a drug (Pippen, 1966) or without signs of previous contact dermatitis (Comaish & Cunliffe, 1967; Roupe & Strannegaard, 1969; Daughters *et al.* 1973).

The two cases described in the present report provide further evidence that percutaneously administered nitrogen mustard may give rise to an anaphylactoid reaction.

Both our patients tolerated nitrogen mustard well and responded excellently to therapy. One patient had received tolerance induction (van Scott & Kalmanson, 1973), the other one had not. Neither had elevated total IgE in serum, as has been found by other workers in patients with mycosis fungoides (Tan *et al.*, 1973) and in a number of our other mycosis fungoides patients (Zachariae *et al.*, 1975).

With all necessary precautions available for treating a severe anaphylactic shock, and giving antihistamines prior to whole body painting, it has been possible to continue the treatment.

REFERENCES

COMAISH, J.S. & CUNLIFFE, W.J. (1967) Absorbtion of drugs from varicose ulcers. A cause of anaphylaxis. *British Journal of Clinical Practice*, **21**, 97.

DAUGHTERS, D., ZACKHEIM, H. & MAIBACH, H. (1973) Urticaria and anaphylactoid reactions after topical application of mechlorethamine. *Archives of Dermatology*, **107**, 429.

ERIKSEN, H.C. (1963) Anaphylactic shock caused by neomycin treated with external cardiac massage. *Ugeskrift for Laeger*, **125**, 1077.

MAUCHER, O.M. (1972) Anaphylaktische Reaktionen beim Epicutantest. *Hautartz*, **23**, 139.

PIPPEN, R. (1966) Anaphylactic reaction after chymacort ointment. *British Medical Journal*, **i**, 1172.

ROUPE, G. & STRANNEGAARD, O. (1969) Anaphylactic shock elicited by topical administration of bacitracin. *Archives of Dermatology*, **100**, 450.

TAN, R.S-H., BUTTERWORTH, C., MCLAUGHLIN, H., MALKA, S. & SAMMAN, P. (1974) Mycosis fungoides, a disease of antigen persistence. *British Journal of Dermatology*, **91**, 607.

VAN SCOTT, E.J. & KALMANSON, J.O. (1973) Complete remissions of mycosis fungoides lymphoma induced by topical nitrogen mustard. *Cancer*, **32**, 5.

WARIN, R.P. & CHAMPION, R.H. (1974) *Urticaria*. Saunders, London.

ZACHARIAE, H., ELLEGAARD, J., GRUNNET, E., SØGAARD, H. & THULIN, H. (1975) T- and B-cells and IgE in mycosis fungoides. *Acta dermato-venerologica*, in press.

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Brief Communication

Generalized pustular psoriasis associated with chronic renal failure

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A male aged 61 years known to be suffering from chronic renal failure due to polycystic kidneys (the latter diagnosed in 1963) was admitted to hospital on 10 March 1975 dyspnoeic and in gross congestive cardiac failure. He gave a 4-week history of dyspnoea and for 2 weeks had had an asymptomatic eruption which had started over the neck and had spread to trunk and limbs. When seen on 12 March he showed extensive erythema with many areas showing pin-point sized sterile pustules either in sheets, rings, or in small patches (Fig. 1). The clinical picture suggested generalized pustular psoriasis and histology (showing spongiform pustules of Kogoj) confirmed this, although the patient gave neither a personal nor a family history of psoriasis. He received peritoneal dialysis in addition to other therapy including bland topical skin applications, but although his skin improved with desquamation within 10 days of admission, he deteriorated generally and died on 31 March.

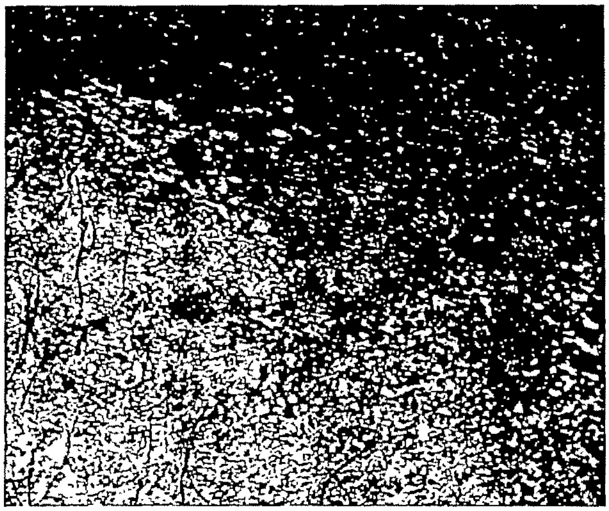

FIGURE 1. Pustules over left thigh.

Investigations. Two months before admission his blood urea was 172 mg/100 ml, serum calcium 9·6 mg/100 ml, serum albumin 3·9 g/100 ml. By 27 March his blood urea had risen to 416 mg/100 ml, with serum calcium 7·8 mg/100 ml, serum albumin 2·4 g/100 ml; the hypocalcaemia is explained by the low serum albumin (to which calcium is bound).

This patient is of interest because his first appearance of psoriasis at the age of 61 was acute and of the generalized pustular form and occurred when he was in terminal renal failure associated with hypocalcaemia. In my opinion the hypocalcaemia precipitated his pustular psoriasis.

Acknowledgment

I should like to thank Dr W.H.R.Cook for asking me to see this patient.

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Comment

TRANSFER FACTOR 1975

It is now 26 years since Lawrence demonstrated that in man transfer of delayed hypersensitivity reactions could be mediated by viable leukocytes, and 20 years since he reported equal efficiency of leukocyte extracts in this situation (Lawrence, 1955). Looking back on these publications at a time when immunology attracts much attention in both popular and professional scientific periodicals, it seems amazing that such observations were not greeted with more interest. In the past decade, however, the realization that the clearly observed benefits of transfer factor (TF) therapy in selected clinical situations lacked a basic background of knowledge of the structure and mode of action of the factor or factors has prompted a brisk interest in basic research into this elusive moiety. This would seem an appropriate time, therefore, to consider the facts currently available concerning its physical and chemical properties and also the clinical conditions in which it has been shown to be of benefit.

Transfer factor is a dialysable non-immunoglobulin structure of molecular weight less than 10,000 and which is resistant to DNAase. Recent work by Gottlieb, Foster & Waldman (1973) on purification of crude TF using a Sephadex G10 Column shows three distinct peaks obtained, one of which demonstrated activity greater than the complete TF preparation on injection into human forearm skin and subsequent challenge with antigen against which the TF donor was known to be sensitized. Further analysis of this component of the buffy coat extract suggested that this active fraction had a molecular weight in the 2000–4000 range, and was likely to be composed of 12 amino acids and 3 or 4 RNA bases. These properties are in contrast to the known features of lymphokines such as migration inhibition factor (MIF) and blastogenic factor which have a molecular weight of 12,000 to 80,000 and are non-dialysable.

The cell type responsible for TF production has not been clearly defined, although observations by Slaven & Garven (1964) suggest that TF is not produced by glass adherent cells. Recently, relatively pure lymphocyte preparations with minimal monocyte admixture have been used for TF preparation, with success.

Despite the remarkable ability of TF in transferring delayed hypersensitivity there are no reported cases of neutralizing anti-TF antibody production in response to its administration. This has been best studied using diphtheria toxoid where extremely sensitive antibody tests are available (Lawrence, 1960). Observations made during clinical application of TF by Good *et al.* (1957) in agammaglobulinaemia and Hitzig *et al.* (1972) in combined immune deficiency confirm this finding; only cellular immunity can be restored and replacement therapy with gamma globulin is still required in these situations.

Two problems which have greatly hampered understanding of the mode of action of TF have been the difficulty in establishing either a reliable animal model, or an *in vitro* assay. There are scattered reports of successful TF activity in primates (Maddison *et al.*, 1972; Zanelli, 1973), but experience with small mammal models has been disappointing, and sporadic reports of successful results have been difficult to confirm. The quantity of cells needed to prepare TF for successful transfer of delayed hypersensitivity to one recipient in small animal models suggests that, if present, TF-like activity in small mammals is very much weaker than in the human situation.

An *in vitro* assay system has proved surprisingly difficult to establish, although Ascher *et al.* (1974) reported some success in the use of antigen specific TF in the conversion of non-immune lymphocytes to immune responsiveness as measured by subsequent antigen mediated lymphocyte transformation. In this situation the uptake of labelled thymidine by lymphocytes cultured with TF is 2–2·5 times that of lymphocytes cultured with antigen alone. The essential requisite for success in this system lies in the preparation of TF; normally TF is dialysed into sterile water, but for the *in vitro* assay dialysis the tissue culture media is essential. The suggested explanation for this is the availability *in vivo* of biological amplification systems not present *in vitro*.

A third problem which remains to be solved is that of specificity; early clinical studies depended greatly on the choice of a TF donor exquisitely sensitive to the antigen to which activity was required in the recipient, but recently a non-specific action has been suggested in the use of pooled TF in such conditions as Behçet's disease where the desired activity is a non-specific 'boost' to cell-mediated immunity, rather than to an identifiable target antigen as in chronic mucocutaneous candidiasis. It seems likely that there may be both specific and non-specific factors at play, and this problem should be solved by the large double-blind trial currently organized by WHO comparing leprosy-specific and coccidiodin-specific TF in the management of both diseases.

There are three main clinical fields in which TF is currently being employed, infectious disease, immunodeficiency, and malignancy. In infectious disease the greatest experience has been reported in the management of chronic mucocutaneous candidiasis, and from a review of the literature it would appear that 60% of reported cases have shown some clinical improvement (Kirkpatrick, Rich & Bennett, 1971; Valdermarsson *et al.*, 1972; MacKie *et al.*, 1976). These patients are a disparate group as is emphasized by careful immunological analysis; the majority have some defect of the cellular immune system and this can be demonstrated by either a negative candida skin test, or a failure of MIF production or a failure of candida-mediated lymphocyte transformation. Clinical improvement is generally preceded by correction of observed *in vitro* abnormalities, and subsequent deterioration by reversal. Patients with chronic mucocutaneous candidiasis in association with endocrinopathy are unlikely to respond clinically to TF administration although *in vitro* changes in cell-mediated immunity may be observed. Other infectious disease states in which TF therapy has been employed with some clinical benefit in approximately half of the treated cases include measles, subacute sclerosing panencephalitis, congenital herpes, coccidiodomycosis and leprosy (Moulias *et al.*, 1973; Bullock, Fields & Brandriss, 1972; Graybill *et al.*, 1973). In many of these case reports, however, there was little *in vitro* monitoring of the immunological status of the patient and studies incorporating this aspect together with adequate long-term follow-up of TF-treated patients are still required.

Congenital immunodeficiency states are an obvious target for TF therapy and in this group, the Wiskott-Aldrich syndrome has received most attention; the largest study has been carried out by Spitler *et al.* (1972) and reports on TF administration to twelve patients with this rare combination of chronic eczema, thrombocytopenia, immunoglobulin abnormalities and susceptibility to recurrent infections. In this situation non-specific TF was given, the donors selected on the basis of a brisk delayed hypersensitivity response to intradermal skin testing with PPD, candida and streptokinase–streptodornase. Six patients thus treated showed recovery from concomitant infection and regression of splenomegaly, five showed improvement of eczema, and three improvement in spontaneous haemorrhage, without, however, any change in the thrombocytopenia. The effect of TF in this situation is said to last for approximately 6 months, when both *in vitro* and *in vivo* parameters revert to the pre-treatment state. TF therapy has also been employed in ataxia telangiectasia with partial clinical benefit (Griscelli *et al.*, 1973) and also in combined immunodeficiency with restoration of CMI but no change in the hypogammaglobulinaemia (Montgomery *et al.*, 1973; Mogerman *et al.*, 1973).

In the management of malignancy TF has been used in advanced breast cancer in five patients using TF from pooled leukocytes of normal females. Although cutaneous reactivity to tuberculin and streptokinase–streptodornase was transferred in three patients, only one showed a partial remission of the disease process (Oettgen *et al.*, 1971). In melanoma Spitler *et al.* (1972, 1973) have administered TF prepared from household contacts of melanoma patients as 75% of these contacts have been shown to be sensitized to melanoma-associated antigen, as measured by MIF production; of nine patients thus treated only one showed tumour regression. TF has also been employed in the management of osteogenic sarcoma and nasopharyngeal carcinoma (Levin *et al.*, 1972; Goldenberg & Brandes, 1972), but results in these situations as in breast carcinoma and melanoma are not at present dramatic, and could well be attributed to spontaneous tumour regression. Further work in a clearly defined trial situation is needed to clarify the role of TF, if any, in the management of malignancy.

It will be seen therefore that there are still vital questions to be answered in the TF field, the most pressing being the cell of origin of TF and its site or sites of activity; one of the reasons for the surprising lack of success of workers in this field in clarifying these two vital points may be the extent to which immunological research in the past decade has been dominated by the lymphocyte; recently this trend has changed and much more attention is now being directed to the monocyte–macrophage system and its role in immunological activity. It may well be that it is in this region that TF exerts its major role and recent work, using a test based on monocyte pinocytosis of radioactive gold developed by Meade, Lachmann & Brenner (1974), suggests that this area is a fruitful one for further investigation. This is urgently needed because, if the clinical value of TF, particularly in chronic infectious disease, is confirmed, then it may well have a major role to play in the eradication of such diseases as leprosy and leishmaniasis which are still major problems in certain areas of the world.

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REFERENCES

ASCHER, M.S., SCHNEIDER, W.J., VALENTINE, F.T. & LAWRENCE, H.S. (1974) *In vitro* properties of leukocyte dialysates containing transfer factor. *Proceedings of the National Academy of Science*, 71, 1178.

BULLOCK, W.E., FIELDS, J.P. & BRANDRISS, M.W. (1972) An evaluation of transfer factor immunotherapy for patients with lepromatous leprosy. *New England Journal of Medicine*, 287, 1053.

GOLDENBERG, G.J. & BRANDES, L.J. (1972) Immunotherapy of nasopharyngeal carcinoma with transfer factor from donors with previous infectious mononucleosis. *Clinical Research*, 20, 947.

GOOD, R.A., VARCO, R.L., AUST, J.B. & ZAK, S.J. (1957) The use of leukocyte dialysate in combined immunodeficiency. *Annals of the New York Academy of Science*, 64, 882.

GOTTLIEB, A.A., FOSTER, L.G. & WALDMAN, S.R. (1973) What is transfer factor? *Lancet*, ii, 822.

GRAYBILL, J.R., SILVA, J., ALFORD, R.H. & THOR, D.E. (1973) Improvement of progressive coccidioidomycosis following transfer factor. *Cellular Immunology*, 8, 120.

GRISCELLI, C., REVILLARD, J.P., BEUTENAL, H. HERZOG, C. & TOURAINE, J.L. (1973) Transfer factor therapy in immunodeficiencies. *Bio-medicine*, 18, 220.

HITZIG, W.H., FONTANELLAZ H.P., MUNTENER, U. *et al.* (1972) Transfer factor. Immunologische Grundlagen und therapeutische Erfarungen. *Schweizerische Medizinische Wochenschrift*, 102, 1237.

KIRKPATRICK, C.G., RICH, R.R. & BENNETT, J.E. (1971) Chronic mucocutaneous candidiasis. Model building in cellular immunity. *Annals of Internal Medicine*, 74, 955.

LAWRENCE, H.S. (1955) The transfer in humans of delayed skin sensitivity to streptococcal M. substance and to tuberculin with disrupted leukocytes. *Journal of Clinical Investigation*, 33, 951.

LAWRENCE, H.S. (1960) Delayed sensitivity and homograft sensitivity. *Annual Review of Medicine*, 11, 207.

LEVIN, A.S., SPITLER, L.E., WYBRAN, J., FUDENBERG, H.H., HELLSTROM, I. & HELLSTROM, K.E. (1972) Treatment of osteogenic sarcoma with transfer factor. *Clinical Research*, 20, 568.

MACKIE, R.M., COCHRAN, R., DE SOUSA, M., PARRATT, D. & ARALA-CHAVES, M. (1976) Chronic mucocutaneous candidiasis treated with transfer factor. *British Journal of Dermatology*, 94, 79.

MADDISON, S.E., HICKLIN, M.D., CONWAY, B.P. & KAGAN, I.G. (1972) Transfer factor. Delayed hypersensitivity to schistosoma mansoni and tuberculin in macaca mulatta. *Science*, 178, 757.

MEADE, C.J., LACHMANN, P.J. & BRENNER, S. (1974) A sensitive assay for cellular hypersensitivity based on the uptake of radioactive colloidal gold. *Immunology*, 27, 227.

MOGERMAN, S.N., LEVIN, A.S., SPITLER, L.E., STITES, D.P., FUDENBERG, H.H. & SHINEFIELD, H.R. (1973) Transfer factor therapy in x linked recessive severe combined immunodeficiency. *Clinical Research*, **21**, 370.

MONTGOMERY, J.R., SMITH, M.A., WILSON, R., RICHIE, E., HEIM, L.R., CRISWELL, S. & TRENTIN, J.J. (1973) Study of a gnotobiotic child with severe combined immunodeficiency. *Clinical Research*, **21**, 118.

MOULIAS, R., GOUST, J.M., REINER, T.P., FOURNEL, J.J., DEVILLE-CHABROLLE, A., DUONG, N., MULLER-BERAT, C.N. & BERTHAUX, P. (1973) Facteur de transfer de l'immunité cellulair. *Nouvelle Presse Medicale*, **2**, 1341.

SLAVEN, R.G. & GARVEN, J.E. (1964) Delayed hypersensitivity in man. Transfer by lymphocyte preparations of peripheral blood. *Science*, **145**, 52.

OETTGEN, H.F., OLD, L.J., FARROW, J.H., VALENTINE, F.T., LAWRENCE, H.S. & THOMAS, L. (1971) Effect of transfer factor in cancer patients. *Journal of Clinical Investigation*, **50**, 71a.

SPITLER, L.E., LEVIN, A.S., STITES, D.P., FUDENBERG, H.H., PIROFSKY, B., AUGUST, C.S., STIEHM, E.R., HITZIG, W.H. & GATTI, R.A. (1972) The Wiskott Aldrich syndrome. Results of transfer factor therapy. *Journal of Clinical Investigation*, **51**, 216.

SPITLER, L.E., WYBRAN, J., FUDENBERG, H.H., LEVIN, A.S., LEWIS, M. & HORN, L. (1973) Transfer factor therapy of malignant melanoma. *Clinical Research*, **1**, 654.

VALDIMARSSON, H., WOOD, C.B., HOBBS, J.R. & HOLT, P.J. (1972) Immunological features in a case of chronic granulomatous candidiasis and its treatment with transfer factor. *Clinical Experimental Immunology*, **11**, 151.

ZANELLI, J., (1973) *Proceedings of Workshop on Basic Properties of Transfer Factor* (Ed. by C.H. Kirkpatrick and J. Rifkind), p. 191. University of Arizona.

British Journal of Dermatology (1976) **94**, 111.

Obituaries

F.J.V.JENNER

Frederick Josiah Victor Jenner died on 18 June 1975, aged 67. He was born in India on 19 March 1908, his father being a civil surgeon in the United Provinces. After qualifying from St Bartholomew's Hospital in 1931 he held house appointments at Charing Cross, the Royal Northern and the London Chest Hospitals. In 1935 he obtained his M.R.C.P. and joined the Middlesex County Medical Service working at Hillingdon, North Middlesex and later West Middlesex Hospitals. While working as a general physician he developed a keen interest in Dermatology and read the subject widely in both English and French. Putting his knowledge to practical use, he started a clinic for skin patients at the West Middlesex and in 1942 was recognized as a specialist dermatologist working at the West Middlesex, Hounslow and Hillingdon Hospitals. When the National Health Service was formed, Fred Jenner made Dermatology his speciality and devoted his whole time to it. He was responsible for providing a service for a population of about 750,000 and his opinion was constantly sought by his medical colleagues.

Apart from his work he was keenly interested in the French language, music and gardening. He is survived by his wife and two daughters.

H.T.H.WILSON

DR HARRY BLACK

It is a sad privilege to write an appreciation of my great and good friend, Harry Black.

Harry died suddenly on 6 September while on holiday with his family in Fiji and we lost a Dermatologist of great excellence. He was a rare combination of the devoted doctor and the dedicated scientist and was a consultant's consultant. Many of us Dermatologists in New Zealand would refer some of our difficult cases to Harry for his valued opinion, such was our regard for his diagnostic and therapeutic knowledge.

Born on 19 October 1919 and educated in Wellington, New Zealand, he spent two years in the public service before yielding to the strong urge to study medicine. He graduated at Otago Medical School in 1945. After two years as House Surgeon in Wellington he was accepted as Registrar in the professorial medical unit at Otago. A further two years saw him feel the challenge of dermatology.

He proceeded to Edinburgh in 1951 where he studied at the Royal Infirmary under Professor Percival. In 1952 he became Registrar in Dermatology under Dr Ingram whose humanitarian and scientific approach to this rapidly developing specialty left a strong and lasting impression. In 1953 he was Senior Dermatological Registrar at St Mary's in London and worked and studied at St John's Hospital for Diseases of the Skin.

His subsequent return to practise as a Dermatologist in Auckland was a great boon for New Zealand. In 1965 he became a Visiting Dermatologist to Auckland Hospital. In 1968 he was Commonwealth Senior Travelling Fellow in Medicine and this took him for six months to important centres of dermatology in Britain, Europe and U.S.A. 1971 saw him become chairman of the Skin Section of Organ Teaching at Auckland Medical School and a censor of the Australasian College of Dermatologists.

As Harry Black's private practice grew larger he was worried about the lessening time available for devotion to his great loves, research and post-graduate teaching. Early in 1975 a new post was created of full-time Dermatologist at Auckland Hospital and to this he was appointed. Some time ago he had established the patch-test clinic and more recently a photo-patch test clinic and was planning eagerly for further organization of the new position.

In 1974 he was elected President of the N.Z. Dermatological Society and, in 1975, Chairman of the N.Z. Faculty of the Australasian College of Dermatologists. He published several papers and took a special interest in the former International Contact Dermatitis Newsletter. Examinations were no great trial to one of his intellect—he achieved the M.R.A.C.P. and M.R.C.P.E. in 1950 and M.A.C.D. in 1966. He was subsequently elected to Fellowship in each of these colleges.

Harry excelled in that hobby appropriate to his profession, namely photography. He could be seen at clinical meetings wielding his latest camera enthusiastically and would project excellent slides at subsequent meetings. He leaves a wonderful collection of photographs of people and nature subjects as well as clinical. Well-versed he was too in music, art and literature. He could well be described as the complete professional man.

Harry Black was one of the modern school of dermatologists and saw the study of skins as a broad concept rather than narrow and he was active in helping it evolve from 'diseases of the skin' to 'disease as it affects the skin'—an important facet of general medicine.

His keen and analytical mind attracted bright young doctors to the specialty and, incalculable though the loss to dermatology is, he has left it vastly enriched.

We grieve deeply and will always remember him fondly. Our heartfelt sympathy goes out to his family who were far and away his greatest love over all, his wife Ruth and his sons Peter and Michael.

M.J.PURDY

This obituary will also appear in the *Australasian Journal of Dermatology.*

Correspondence

WITHDRAWAL OF ZINC THERAPY IN ACRODERMATITIS ENTEROPATHICA

SIR, Following our case report of a girl aged 16 years with acrodermatitis enteropathica (AE) successfully treated by oral zinc (Portnoy & Molokhia, 1974), it was decided to stop zinc supplements in order to pin-point zinc as a specific factor in clearing this condition.

Zinc sulphate capsules were substituted by a placebo for a period of 20 weeks during which the patient was examined at regular intervals. The only abnormality which became apparent was slight roughness of the skin of the backs of the hands. The fact that there was no recurrence of the disease during that period seemed inexplicable. The possibility that the patient had built up a sufficient body store of zinc was unlikely in view of the relatively high turnover rate of zinc and the lack of any proven depot for that metal in the body (Davies, Musa & Dormandy, 1968).

The lack of any abnormality at that time suggested that zinc metabolism was following a normal pattern and that the patient was receiving adequate supplies from the diet alone. However, estimation of zinc levels in serum at the end of the 20 weeks showed a reduction, from 14·80 during zinc therapy to 4·33 ppm (dry weight).

At the end of week 20, a further challenge was presented in the form of oral calcium. High calcium intake is known to reduce the availability of dietary zinc and produce a zinc deficiency syndrome in animals (Underwood, 1971). Effervescent calcium tablets in a dose equivalent to 1·5 g of calcium daily were given to the patient. Within 10 days new lesions started to appear on the face and, in particular, around the mouth and on the chin. These lesions were papulo-pustular and were soon found to coalesce into psoriasiform plaques. At the same time, the patient complained of general fatigue and joint pains in her feet.

In the following week, she developed new skin lesions on her forehead and nape of the neck and a mild degree of paronychia was detected in one of her fingers. She was found to be lethargic and maintained a depressed facial expression. Analysis of serum at that stage showed a zinc level of 8·05 ppm.

Zinc sulphate therapy, 450 mg daily, was commenced once more and within 7 days the whole situation had changed. The facial lesions completely cleared and hand roughness disappeared. She no longer complained of arthralgia in her feet. This dramatic development was accompanied by a complete change in mood and a happy facial expression. The paronychia, however, persisted for 3 weeks before it finally disappeared.

This account shows that zinc is directly involved in clearing as well as in causing this hereditary condition. It also emphasizes the role which might be played by high calcium intake in precipitating the disorder in susceptible individuals. The dose of calcium given to the patient was not more than the daily requirements of a person of her age yet this seemed to be enough to cause a shift in the balance which existed. We speculate that a similar situation exists in babies who are weaned on to cow's milk and that this could be a decisive factor in the appearance of acrodermatitis lesions at that stage. Cow's milk is known to be rich in calcium. Standard milk preparations used for feeding babies were found to contain 2–3 times more calcium compared with human milk (Baum & Harker, 1975).

This is the first time a challenge of this nature has been made in a human and a return to normality obtained by the exhibition of zinc. We believe it is a remarkable example of how a metabolic disorder can be reversed simply by altering the mineral environment.

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REFERENCES

BAUM, J.D. & HARKER, P. (1975) National dried milk. *Lancet*, **i**, 159.

PORTNOY, B. & MOLOKHIA, M. (1974) Acrodermatitis Enteropathica treated by zinc. *British Journal of Dermatology*, **91**, 701.

DAVIES, I.J.T., MUSA, M. & DORMANDY, T.L. (1968) Measurement of plasma zinc. I. In health and disease. *Journal of Clinical Pathology*, **21**, 359.

UNDERWOOD, E.J. (1971) In: *Trace Elements in Human Nutrition*, Chapt. 8. Academic Press, New York.

URTICARIA AND HYPERTHYROIDISM

SIR, I agree with your contributors, Drs Pace & Garretts in the July number of the journal that thyrotoxicosis may present with urticaria, and I have recorded a case (Lyell, 1972). I discovered from Barrow & Bird (1966) that Sir William Osler had described the association, and I confirmed this (Osler, 1892). Thyrotoxicosis may also cause pruritus without urticaria. These occurrences are well documented but are not well known. It may be that they are commoner than is generally realized due to the reluctance of physicians to see any connection between itching and thyrotoxicosis.

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REFERENCES

BARROW, M.V. & BIRD, E.D. (1966) Pruritus in hyperthyroidism. *Archives of Dermatology*, **93**, 237.

LYELL, A. (1972) The itching patient: a review of the causes of pruritus. *Scottish Medical Journal*, **17**, 334.

OSLER, W. (1892) *The Principles and Practice of Medicine*, p. 714. Pentland, Edinburgh.

Book Reviews

The Biology of Racial Integration. K.F.DYEK (1974) Bristol: Scientechnica (Publishers) Ltd. Pp. xiii+446, illustrated. Price £12.50.

It is said that all the differences between individuals of different races are determined by some 10% of the total complement of genes of *Homo sapiens*. These differences include notably the conspicuous morphological variations, and variations in the incidence of obviously hereditary diseases, but also variations in susceptibility to environmental hazards, microbial, physical and chemical. The importance of increasing our scanty knowledge of such variations needs no emphasis, yet in planning investigations clinicians seldom seek the advice of anthropologists and tend to ignore the accepted quantitative criteria, and the nomenclature that the latter employ.

Dr K.F.Dyer of Monash University, Australia, has taken as the central theme of his book, the unquestionable assertion that 'the history of man is the history of racial integration'. The broad racial categories—Mongoloid, Caucasoid, Negroid or Australoid—in which clinicians classify their patients, if they classify them at all, have little biological significance. Dr Dyer quotes recent interesting work on microraces, which should perhaps be the units for clinical studies. The Welsh are an example of a microrace, yet even within Wales there are important differences in gene distribution; the high incidence of blood Group A in South Pembrokeshire supports the accepted tradition of Viking settlement in this area. The numerous microraces of continental Europe owe their differences partly to successive waves of immigrants and partly to differentiation within local communities in response to different environmental conditions.

That the use of the very broad categories may be misleading is shown by the striking differences in the inheritance of hair form in crosses between Negroids in the United States (probably of West African origin) and Caucasoids of European origin, as compared with crosses between Negroids (Zulus) and Caucasoids of Indian origin. These differences emphasize the complexity of the genetic control of hair form and are a warning against premature generalization.

Topics considered in this book include the history of the races of man, the biology of race crosses (heterosis and coadaptation) and the genetics and practical consequences of racial integration. Pigmentation and hair form are discussed at some length, as are the striking racial differences in the incidence of many diseases—such as, for example, the various types of albinism. The book can be recommended to the dermatologist who is interested in the wider biological implications of his specialty, and most particularly if he is planning incidence studies of disease.

ARTHUR ROOK

Progress in Allergy. Edited by P.KALLÓS (Helsingborg), B.H.WAKSMAN (New Haven, Conn.) and A.DE WECK (Bern) (1975) vol. 18. Pp. x+477, 46 illustrations, 38 tables. Price £27.20.

A further volume in a series of reviews which have maintained a consistent high standard. The scientific detail is much greater than would be required by some clinicians and reflects the current interest in lymphocytes and the early stages of the induction of an immune response. It contains, however, a well-presented account of 'Immunological aspects of urinary tract infections', and a timely review of 'cross-reactive antigens and mammalian tissues' with particular reference to streptococcal antigens in auto-immunity, though there is a disappointingly small section (1 page) on cross-reactive antigens and problems of vaccination. Further information on auto-immunity is presented in chapters on 'Glycolipid haptens of myelin', 'Antigenic competition', and 'Immunological tolerance'.

There is much valuable information in all chapters for research workers, and a comprehensive (23 pages of references) though slightly unreadable review of the 'Structure and metabolism of lymphocyte membranes'. Further information on lymphocytes and properties of their membranes is available in a more critical account of present knowledge on 'Antigen recognition' in 'Induction of immunity'.

This book should be available to all those seriously interested in the initial response to antigenic recognition and stimulation, and the activities of lymphocytes.

W.E.PARISH

The Practitioner's Dermatology. R.L.SUTTON JR and M.WAISMAN (1975) New York: Yorke Medical Books. Pp. xiv+521, illustrated. Price $30.00.

In 1939 R.L.Sutton Jr collaborated with his father to produce the 10th edition of *Diseases of the Skin.*

Now, well over 30 years later he has himself collaborated with a younger man to produce a comprehensive textbook of dermatology. The result is a delightful résumé of modern dermatological teaching written in a concise yet most refreshing style. This may be illustrated by the paragraph on the hazards of methotrexate therapy for psoriasis which concludes 'When things go well in a patient stabilized on the drug, a few pills at intervals of a week or more keep the disease largely out of sight—the patient delighted, the physician edgy!'

The happy wedding of a life-time of clinical experience with an up-to-date approach to immunology and pharmacology has been achieved by the two authors having somehow managed to resolve their 'diametric disagreements'. Sutton admits he is 'archaic enough to mourn the dying art of dermatologic radiotherapy and irreverent enough to disbelieve most of the clamour of the fruit-fly cognoscenti', whereas Waisman is 'conservative, party-line and safe'. They had originally intended to include only what they both believed, but this proved impossible, and in places they have had to insert (W) which means Waisman thinks this, but Sutton does not, or (S) which means Sutton believes this, but Waisman does not. This device is less disruptive than one might expect, but occasionally it produces sentences such as—'Used as a curette the fingernail is an instrument that lends itself to virtuosity (S), but its use is inelegant and unsanitary (W)!'

The book is midway in size between the small undergraduate texts and the reference tomes. It includes everything I would expect a good general practitioner or registrar to know and it would be excellent for MRCP candidates. It contains some facts which are not yet to be found in the larger books (e.g. diazoxide causes hypertrichosis) and it claims a smattering of original observations (e.g. active morphoea is warm to the touch). There are few controversial statements which are not protected by the (S), (W) device, and one has to search carefully for points of contention. Does congestive cardiac failure cause itching due to inadequate blood flow (p. 4)? Do hot flashes (*sic*) of the climacteric affect men as well as women (p. 277)? Some of the treatments die hard in Prairie Village, Kansas (e.g. Vleminckx's solution for acne) and I doubt if dietary restriction of chocolate, nuts, cola drinks, alcohol, ice-cream and oily cheese appeals to patients with acne as much as the authors claim, but they do have the grace to explain that transgressions from these restrictions allow the physician to provide a self-righteous explanation of the recalcitrance of the disease.

The book is beautifully produced, with an adequate index and over 600 black and white photographs, which are small but of good quality. I can heartily recommend it.

J.L.BURTON

Spezielle Pathologische Anatomie, Vol. 9. Pathologie des Ohres. W.SCHÄTZLE and J.HAUBRICH (1975) Berlin, Heidelberg, New York: Springer. Pp. 258, 129 illustrations. Price £24.00.

This is a further volume in the comprehensive series edited by Doerr, Seifert and Uehlinger, of which the dermatological section in Vol. 3 has already been reviewed in this Journal (August, 1975).

It is divided into three parts devoted respectively to the external, middle and inner ear; of these the first is naturally of most interest to dermatologists. It occupies 51 pages and deals with developmental anomalies, injuries, foreign bodies, inflammations and tumours in that order. It appears to be a comprehensive and up-to-date account of knowledge in this area of otological and dermatological overlap and is therefore a useful source of information and reference.

It is possible to indicate omissions: for example, there is no mention of the evidence in favour of a vascular anomaly in chondrodermatitis nodularis helicis. The entity referred to as 'panchondritis rheumatica' is more familiar to us as 'relapsing polychondritis' for which one would seek in vain in the index. The distinction between keratoacanthoma and squamous-cell carcinoma can pose a therapeutic dilemma in this site but the former lesion receives only a six-line paragraph. And is leiomyoma of the ear only fortuitously encountered?

The cited literature occupies 27 pages and covers all three divisions of the book. Although naturally biased in favour of German publications, it is international in scope.

P.J.HARE

Haut und Anhangsgebilde: Spezielle Histopathologie (Band 7 of Spezielle Pathologische Anatomie). Edited by U.W.Schnyder (1975) Berlin: Springer Verlag. Pp. 793, 476 illustrations.

The publication of a new substantive text on the histopathology of the skin is a notable event and fills a gap in the present German medical literature. This is a beautifully produced book with fine illustrations (albeit rather expensive) in the well known tradition of Springer Verlag.

The text itself is in some ways disappointing as there is little attempt to break new ground, and the overall impression is of a cautious and conventional approach. The way individual conditions are grouped and classified reflects the older European tradition. For instance the chapter on dermo-epidermal conditions is a hotch-potch, with miscellaneous entities such as xeroderma pigmentosum, lichen planus, perforating elastoma, parapsoriasis, miliaria, chondrodermatitis helicis and lichen sclerosus, among others thrown together. Thus scleroderma becomes quite separated from lichen sclerosus as it is discussed in a separate chapter; and, would it not have been more suitable to deal with parapsoriasis with the problem of the reticuloses?

In the preface the hope was expressed that clinical physiological aspects and electron microscopy, etc. would be fully integrated into a modern appraisal of this subject. In the event most of the individual authors do not step outside traditional histopathology, and recent advances in immunofluorescence are hardly mentioned. An exception to this is an excellent chapter by Rupec on the Ultrastructure of the Epidermis. This chapter is up-to-date and contains numerous references to current work. One wonders why the consideration of electron microscopy is limited to the anatomy of the epidermis, and if in fact a more ambitious scheme had been planned but had not come to fruition. Most of the other authors appear to have taken much less trouble to see their references are relevant and really up-to-date—or was it that some of the chapters had sat on ice for a long period awaiting the printing? As an illustration of this, it is surprising to find Mach in his chapter on Reticulosen und Granulomatosen quoting a 1958 reference which implies that about three-quarters of the blood lymphocytes have their origin from the germinal centres of the lymph nodes—so much for the B and T cell explosion of the last 8–10 years! Surely it is now well known that 60–80% of the blood lymphocytes are T cells, and these cells are related to the paracortical areas of the nodes and not the follicles?

In spite of these criticisms this book has many fine points and I really have enjoyed reading it in detail, but I recognize that this text would be of limited appeal to dermatologists as a whole, except for those who can read German easily or who aspire to be professionals at skin pathology.

E.Wilson Jones

The modern guide to skin care and beauty. I.I.Lubowe (1975) London: George Allen and Unwin. Pp. 224, illustrated. Price £4.25.

Although now published in London this book is written by an American, in American, and one would have thought for the American lay public. Certainly it deals with American life rather than the British counterpart.

The author describes the structure and functions of the skin, and then has chapters on topics as various as sun on the skin, cosmetics for men and women, pigmentation, dandruff, diet, ageing changes (including the modish subject of long-term hormone replacement at the menopause) and detailed advice on how and what to pack for a holiday. Acne has a chapter to itself; other skin disorders, an arbitrary selection, are mentioned in a total of five pages, a good opportunity for public education in the fields of, for example, psoriasis and eczema being thereby missed.

Dr Lubowe rightly stresses the danger of light on the skin and of allergy to various products, and cautions against the pressures of commercialism. Some of his views would not be generally accepted in this country—for example, his advocacy of vitamin B_{12} in seborrhoea and his implication of excess fat in the diet as a cause of excessive sebum production. Dog-loving readers may be saddened to learn that though they may avoid the Scylla of papular urticaria by fitting their pet with a flea collar they may then be drawn into the Charybdis of a contact dermatitis from the insecticide.

It is difficult to see a useful role for this book to play; there already exist good short books on the skin for the enquiring lay person, and advice on beauty care and kindred topics of dermatological relevance are in general quite responsibly covered by women's magazines.

C.M.Ridley

Skin Microbiology Club

The club held a joint meeting in January 1975 with the Pathology Society of Great Britain and Ireland at the Middlesex Hospital Medical School. The papers for the symposium and the open session are published in abstract in *Journal of Medical Microbiology*, **8,** No. 2, 1975. The sixth summer meeting of the club was held at the Westminster Medical School. Laboratory demonstrations on technique were followed by papers on interactions between skin bacteria and the isolation and characterization of an antibiotic from *Staphylococcus epidermidis.* A glimpse at the antigenic structure of stratified squamous epithelium was also obtained.

British Journal of Dermatology (1976) **94**, 118.

News and Notices

Annual Meeting of B.A.D. 1976

The Annual Meeting of the British Association of Dermatologists will take place in London on 8, 9 and 10 July 1976, under the presidency of Dr David Williams. There will be opportunities for additional papers, of a laboratory orientated nature, on the Friday afternoon for those who do not wish to join in the social and sporting activities. The rest of the programme will be as usual. Papers are invited and should be sent in the form of a summary of approximately 200 words (6 copies) to the Honorary Secretary, Dr K.V.Sanderson, British Association of Dermatologists, 1 Wimpole Street, London W1M 8AE, before 1 March 1976. If there is more than one author, please underline the name of the speaker. The British Journal of Dermatology will publish in a supplement the summary of those papers which are accepted. These, therefore, should be detailed, precise and explicit and avoid such phrases as 'the results will be discussed'. Any references should be set out as required by the British Journal of Dermatology.

First International Symposium on HLA and Disease

23–25 June, 1976. Paris, Palais des Congrès.

The association of HLA antigens with numerous diseases is now well documented. The clinical implications of these recent discoveries on nosology, diagnosis, prognosis and even prevention will be discussed by clinicians and biologists.

Several workshops (rheumatology, dermatology, gastroenterology, allergy, immuno-pathology, endocrinology) will cover the different facets of this new approach to epidemiology and hereditary diseases. The possible mechanisms and biological significance of association will be considered by geneticists, virologists, and immunologists. Proceedings of the symposium will be published by Munksgaard, Copenhagen. For further information, please contact:

Congrès Services,
1, Rue Jules Lefebvre,
75009 Paris,
France.

Advanced Course in Dermatology

This seminar is open to Consultants and Senior Registrars in Dermatology. It will be held from 5 to 9 April 1976 at the Institute of Dermatology and St John's Hospital for Diseases of the Skin, Lisle Street, Leicester Square, London WC2H 7BJ. Applications should be sent to the Dean before 31 January 1976.

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm³	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine London.

Illustrations should be referred to in the text as 'Figs.', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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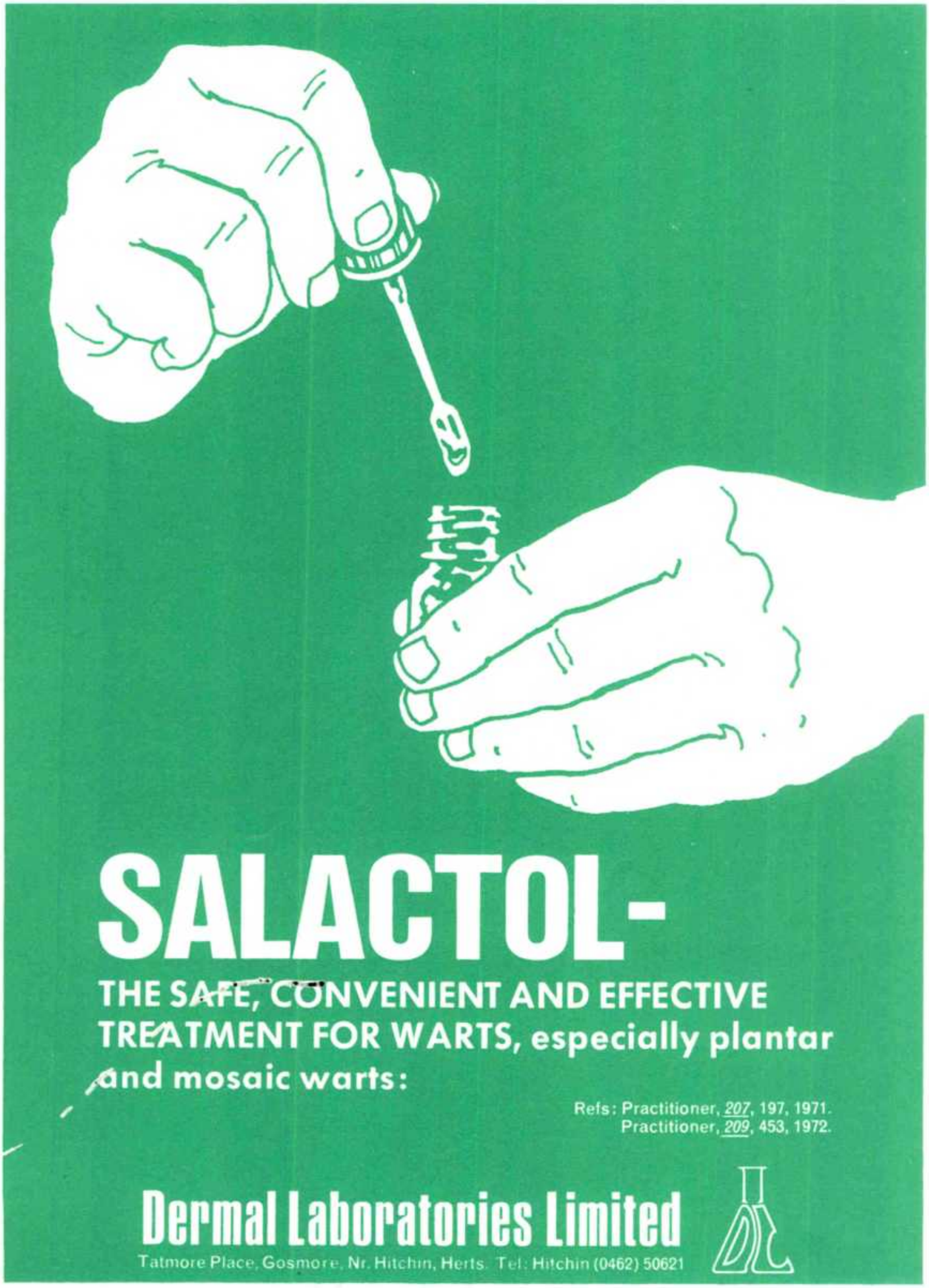

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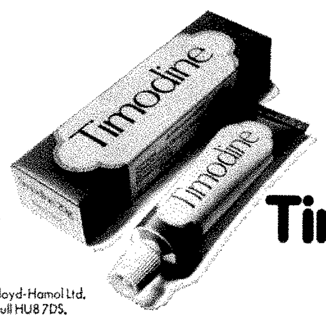

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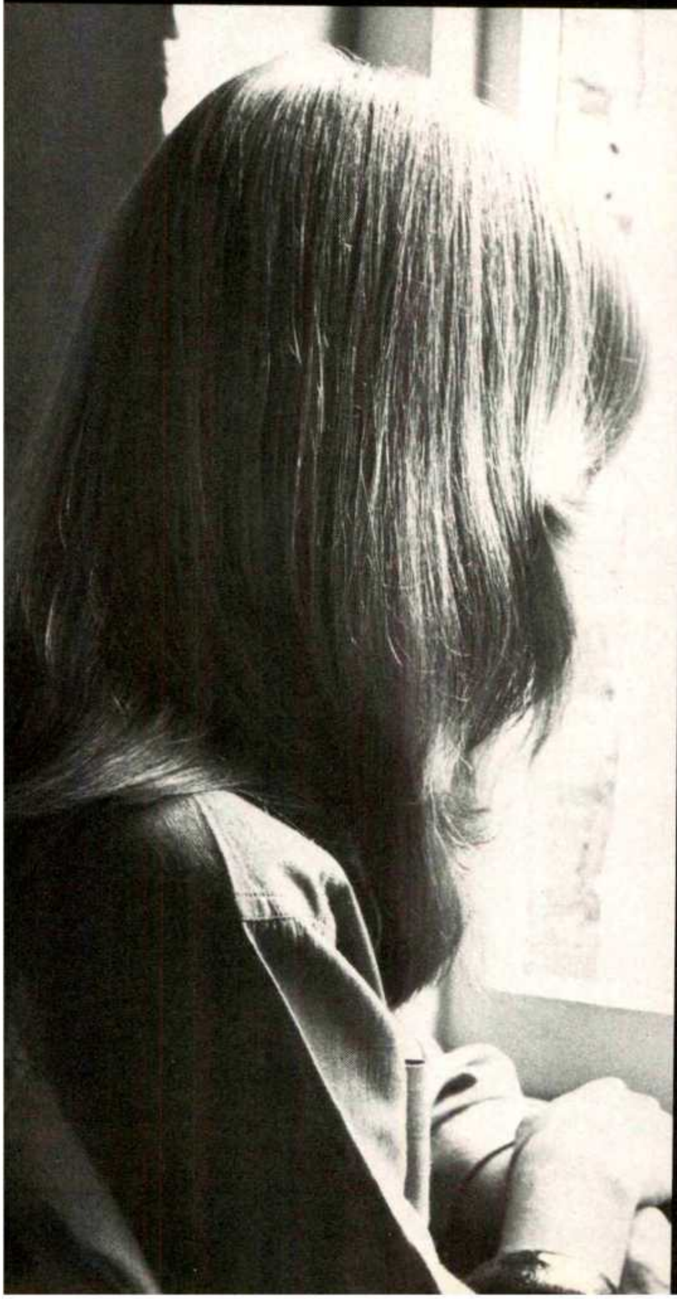

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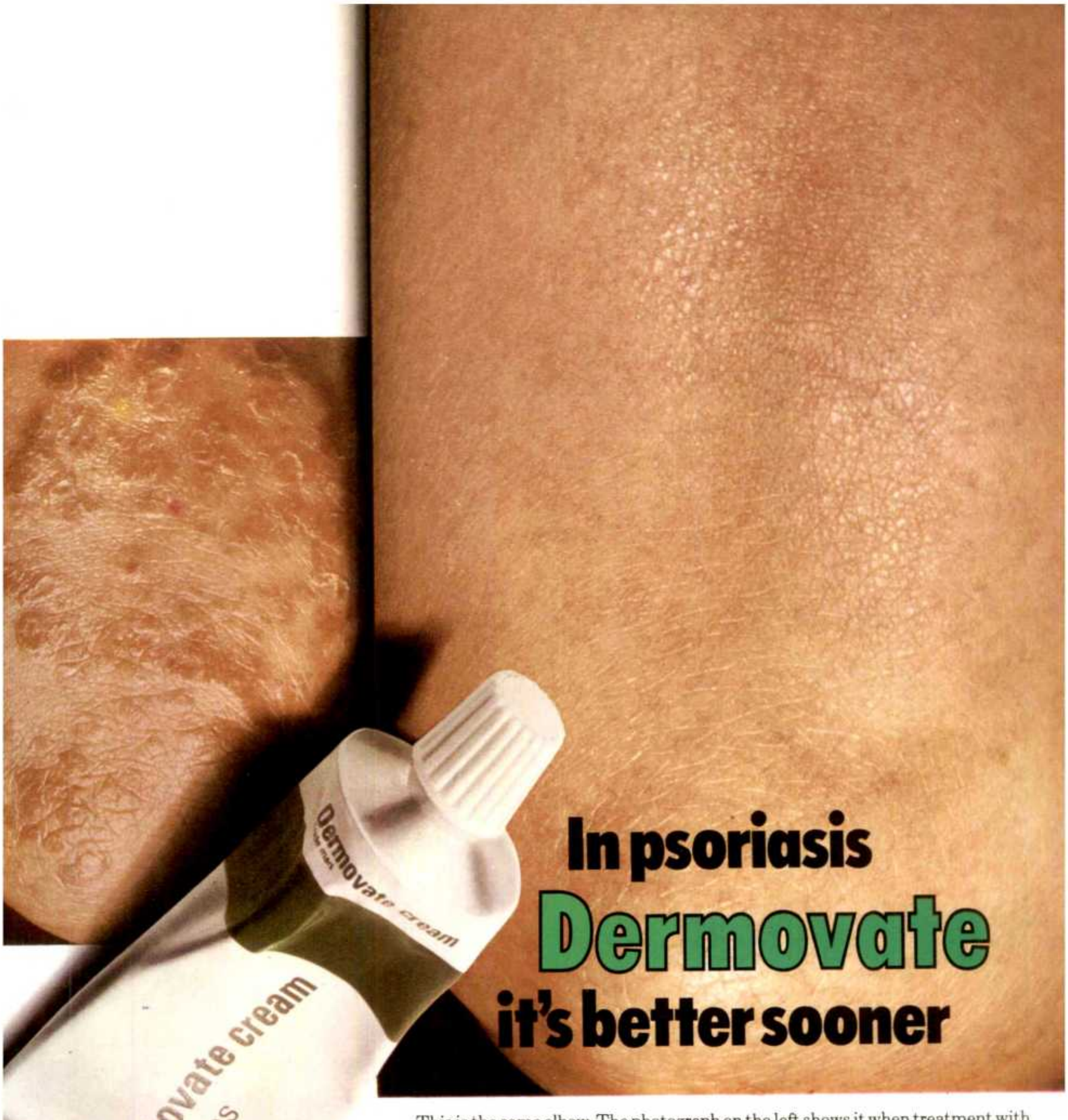

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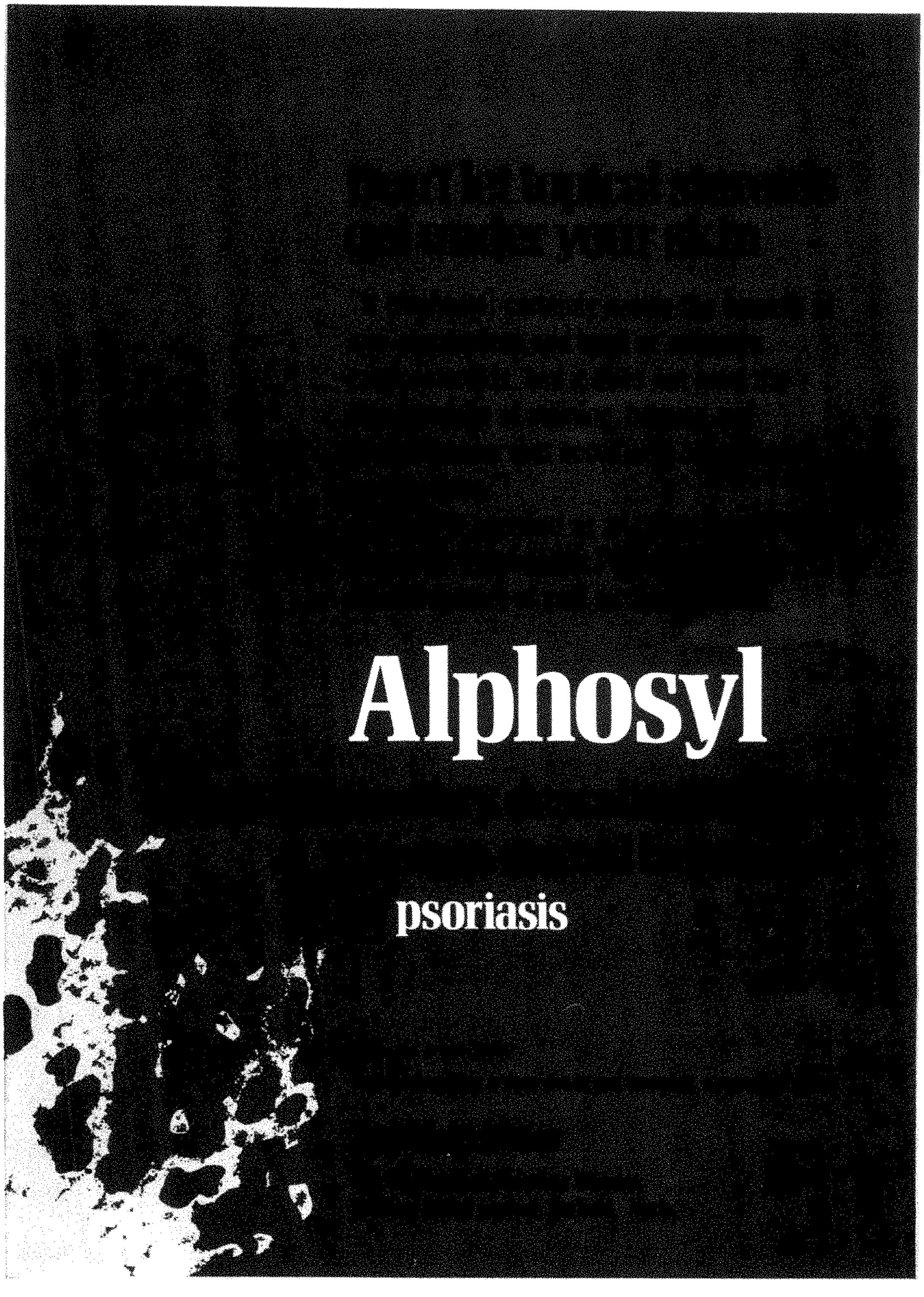

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HISTOPATHOLOGY OF THE SKIN

Walter F. Lever M.D. and Gundula Schaumburg-Lever M.D. *Fifth Edition*, 1975. 816 pages, 436 illustrations (8 colour). Lippincott, £36.40

In the eight years that have passed since publication of the fourth edition so much new information has become available on nearly every major dermatosis that the book had to be entirely rewritten. The greatest changes were required in the description of the lymphomas because they have been reclassified in recent years. Many changes have been made also in the description of the bullous dermatoses and of the metabolic diseases, especially of amyloidosis, colloid milium, hyalinosis cutis et mucosae, and porphyria. Also the division of malignant melanoma in situ into two types with different prognosis has been given due consideration.

More than 20 diseases not mentioned in the fourth edition are described in this edition. Some of them are newly recognized entities, such as disseminated superficial actinic porokeratosis, perforating folliculitis, reactive perforating collagenosis, angiolymphoid hyperplasia with eosinophilia, infantile digital fibromatosis, trichilemmoma, and malignant eccrine poroma. Also several previously known diseases because of their increased interest for the dermatologist have been incorporated, among them regional enteritis (Crohn's disease), Tangier disease, lipogranulomatosis (Farber's disease), and generalized eruptive histiocytoma.

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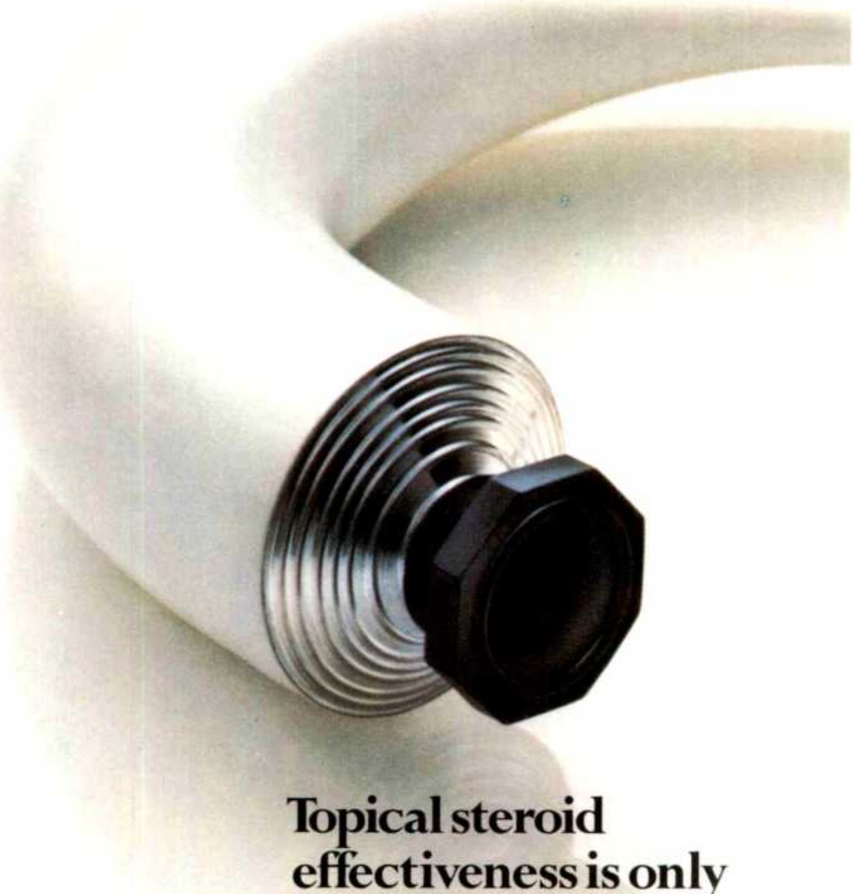

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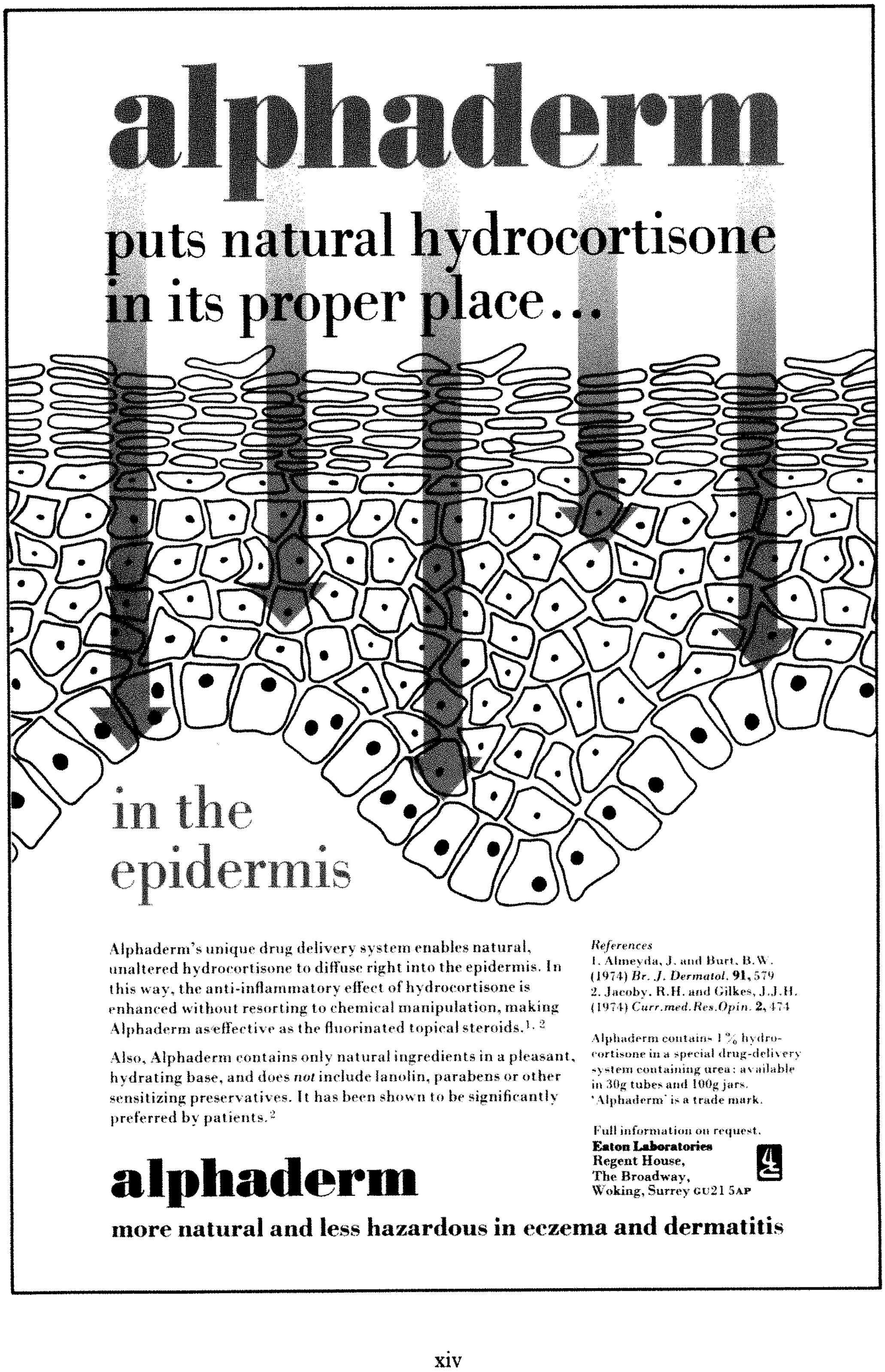

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References
1. Almeyda, J. and Burt, B.W. (1974) *Br. J. Dermatol.* **91**, 579
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Clinical and Laboratory Investigations

Langerhans cells in the epidermis of athymic mice

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SUMMARY

Langerhans cells were identified with the electron microscope in the epidermis of six athymic *nu/nu* mice. Their origin in the thymus is ruled out and their functional dependence on either the thymus or T lymphocytes seems improbable.

The origin and function of the epidermal Langerhans cell are still unknown. Apparently identical cells occur in extra-epidermal situations including lymph nodes (Jimbow, Sato & Kukita, 1969; Kondo, 1969) and the thymus (Haelst, 1969), and since Wolff's recent comprehensive review of the cell (Wolff, 1972) attention has been drawn to the possibility of an immunological role for the cells in contact allergy (Silberberg, Baer & Rosenthal, 1974). It therefore seemed worth while to find out whether Langerhans cells were present in the epidermis of athymic mice, in which T lymphocytes are virtually absent and delayed hypersensitivity reactions, including contact allergy, are abolished (Pantelouris, 1973).

MATERIALS AND METHODS

The mice were three black and three albino male nude homozygotes (*nu/nu*), 6–7 weeks old, raised under specific pathogen-free conditions at the Moredun Institute for Animal Diseases, Edinburgh. Mice from this stock have the typical nude phenotype (Flanagan, 1966) and accept grafts of human skin (Cubie, 1972). Post mortem examination of each mouse confirmed the absence of a thymus.

The mice were killed by cervical dislocation and pieces of mid-dorsal skin were immediately immersed in half-strength Karnovsky's fixative (Karnovsky, 1965), cut into small blocks and fixed for 5 h at room temperature. After post-fixation in 1% aqueous osmium tetroxide, specimens were stained *en bloc* with 2% aqueous uranyl acetate, and embedded in araldite. Thick sections stained with toluidine blue were examined and thin sections of selected areas of epidermis containing dendritic cells were cut with an LKB III ultra microtome. Sections on grids were stained with uranyl acetate and lead citrate and examined using an A.E.I. Corinth at 50 kv.

RESULTS

Epidermal Langerhans cells were found without difficulty in each of the six mice (Fig. 1). In one mouse a Langerhans cell was seen in the dermis. The granules in the Langerhans cells appeared normal;

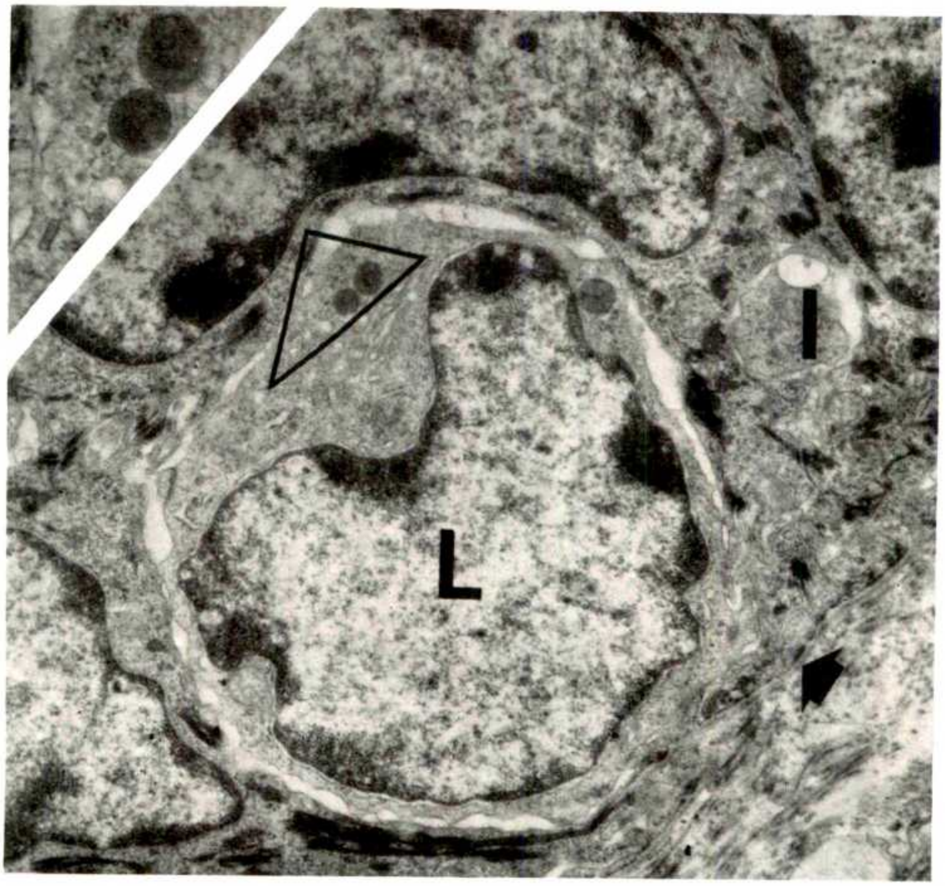

FIGURE 1. Langerhans cell (L) and its dendritic process (l) between keratinocytes of the basal cell layer. Arrow points to basal lamina (× 13,500). Inset (area outlined in triangle) shows a granule and two lysosomes (× 35,000).

both rod and racket shaped profiles were noted (Fig. 2) and one was seen in contact with the cell wall. Other intracellular organelles including mitochondria, ribosomes, Golgi and endoplasmic reticulum were normal, though lysosomes were particularly numerous in some cells. Centrioles were also seen. The appearance of the Langerhans cells therefore indicated an active cell population. The ultrastructure of the rest of the epidermis, although not studied in detail, seemed unremarkable.

COMMENT

The origin and function of the epidermal Langerhans cell are still unknown (Wolff, 1972). Wolff favoured a mesodermal origin. Silberberg *et al.* (1974) suggested an immunological function in contact allergy reactions, where a close relationship with mononuclear cells (in this case, T lymphocytes) is envisaged and the Langerhans cell might process antigen like the macrophage.

Cells containing Langerhans granules have been noted in the thymus (Haelst, 1969), an organ which produces and influences the function of T lymphocytes. If there is a close relationship between Langerhans cells and T lymphocytes, as suggested by Silberberg, Langerhans cells could also originate

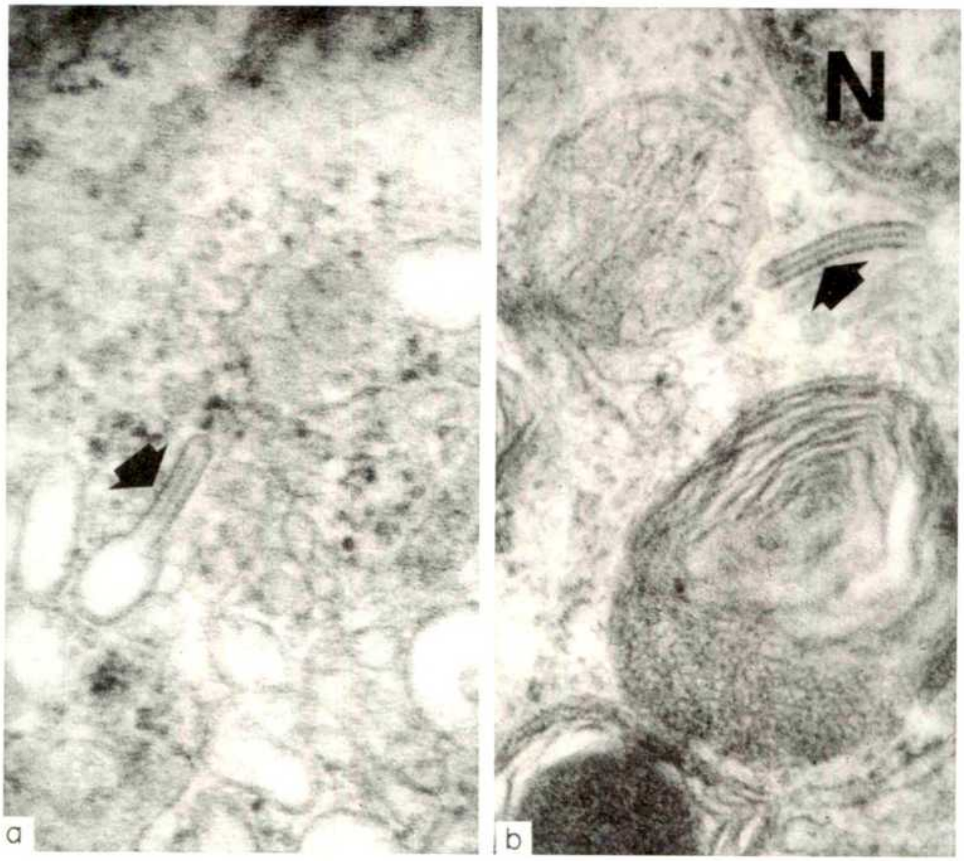

FIGURE 2. (a) Racket shaped granule (arrow) (×92,000). (b) Rod shaped granule (arrow) below which are seen two lysosomes. N, nucleus (×81,500).

in the thymus, or be dependent on it. Langerhans cells appear in human embryonic skin at 14 weeks (Breathnach & Wyllie, 1965) while the thymus develops earlier at about 9 weeks (Hamilton, Boyd & Mossman, 1972), so a thymic origin for Langerhans cells is possible.

In nude mice the thymus never reaches the lymphoid state and remains as a cystic rudiment. As a consequence immunological competence is not achieved (Pantelouris, 1973). Circulating lymphocytes are reduced 5–6 fold and at least 97% of those remaining are B cells (Sprent & Miller, 1972). In fact many nude animals appear to have no T cells at all when examined by the sheep red blood cell rosette test. Delayed hypersensitivity reactions, including contact dermatitis, are abolished. Grafts of foreign tissue are accepted.

We believe that the finding of Langerhans cells in athymic mice shows that the epidermal Langerhans cell: (1) does not originate in the thymus, and (2) on structural evidence alone, is not dependent on the thymus or T lymphocytes.

This does not preclude a close functional relationship between T cells and Langerhans cells, as suggested by Silberberg *et al.* (1974).

It will be important to determine whether the Langerhans cell population in athymic mice differs in size from that in normal mice. Histochemical studies with that aim are in progress.

ACKNOWLEDGMENT

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REFERENCES

BREATHNACH, A.S. & WYLLIE, L.M.A. (1965) Electron microscopy of melanocytes and Langerhans cells in human fetal epidermis at fourteen weeks. *Journal of Investigative Dermatology*, **44**, 51.

CUBIE, H.A. (1972) Laboratory investigations in the study of human papilloma virus. *M.Sc. thesis*, University of Edinburgh.

FLANAGAN, S.P. (1966) 'Nude', a new hairless gene with pleiotropic effects in the mouse. *Genetic Research*, **8**, 295.

HAELST, U.J.C. VAN (1969) Light and electron microscopic study of the normal and pathological thymus of the rat. III. A mesenchymal histiocytic type of cell. *Zeitschrift für Zellforschung und mikroskopische Anatomie*, **99**, 198.

HAMILTON, W.J., BOYD, J.D. & MOSSMAN, H.W. (1972) *Human Embryology*, 4th edn, p. 319. Heffer, Cambridge.

JIMBOW, K., SATO, S. & KUKITA, A. (1969) Cells containing Langerhans granules in human lymph nodes of dermatopathic lymphadenopathy. *Journal of Investigative Dermatology*, **53**, 295.

KARNOVSKY, M.J. (1965) A formaldehyde–glutaraldehyde fixative of high osmolarity for use in electron microscopy. *Journal of Cell Biology*, **27**, 137A.

KONDO, Y. (1969) Macrophages containing Langerhans cell granules in normal lymph nodes of the rabbit. *Zeitschrift für Zellforschung und mikroskopische Anatomie*, **98**, 506.

PANTELOURIS, E.M. (1973) Athymic development in the mouse. *Differentiation*, **1**, 437.

SILBERBERG, I., BAER, R.L. & ROSENTHAL, S.A. (1974) The role of Langerhans cells in contact allergy. *Acta dermato-venereologica*, **54**, 321.

SPRENT, J.R. & MILLER, J.F.A.P. (1972) Thoracic duct lymphocytes from nude mice: migratory properties and life span. *European Journal of Immunology*, **2**, 384.

WOLFF, K. (1972) The Langerhans Cell. In: *Current Problems in Dermatology*, Vol. 4 (ed. by J.W.H. Mali), p. 79. Karger, Basel.

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Necrotizing vasculitis: a circulating immune complex producing inflammatory skin lesions

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SUMMARY

A patient with acute necrotizing vasculitis is described in whom tests for circulating immune complexes were negative. The patient's serum injected intradermally produced lesions which closely resembled those occurring spontaneously. When the serum was fractionated, the skin-reactive fraction was found to be associated with IgG, but was of a higher molecular weight than normal IgG. Immunofluorescent studies showed that lesions induced by this active fraction contained IgG and complement in the epidermal basement membrane zone and within the small dermal vessels. We conclude that an immune complex of relatively low molecular weight is present in the active fractions, and capable of initiating the lesions of acute necrotizing vasculitis.

Acute necrotizing vasculitis (Milne, 1972) (leukocytoclastic angiitis) is a rare skin disease of adults which may also affect other organs. There is a widespread erythematous macular or papular eruption sometimes including elements of purpura, urticaria, pustules and nodules. Fibrinoid necrosis is found in the small vessel walls, with a heavy inflammatory infiltrate of neutrophils, many of which show pyknosis and nuclear fragmentation. In some cases, immunoglobulins and complement can be detected in the lesions (Parish, 1970). There is indirect evidence for the presence of immune complexes in the circulation in some cases: Cream (1973) reported a high level of anti-complementary activity in one patient with a similar vasculitis, and Parish (1970) in a study of fifty-six cases, found eleven with rheumatoid factor and six with immuno-conglutinin (Parish, 1970, 1971). However, Parish (1971) concluded 'It has not been established that cutaneous vasculitis is induced by complexes'.

We describe studies on a patient with acute necrotizing vasculitis in whom all available tests for circulating immune complexes were negative, but whose serum has been shown to contain an IgG-related component (presumably an immune complex) which reproduces the skin lesions on intradermal injection.

CASE REPORT

J.W., a 61-year-old clerk, was first seen in a dermatology clinic in 1972, with a 6-months history of a widespread recurrent rash distributed on his arms, the back of his neck, trunk and buttocks. There was also malaise, arthralgia and weakness, the whole having begun as a 'flu-like' illness. The lesions were erythematous macules and papules together with small cutaneous nodules; they were not tender, and did not itch. There was no history of drug exposure and topical steroids had been used without effect. Clinically, the lesions were thought to be those of a vasculitis. A biopsy showed classical acute necrotizing vasculitis (Fig. 1). On the left thigh there were two firm and tender subcutaneous plaques. Biopsies showed the changes of chronic fibrosing panniculitis without evidence of vasculitis.

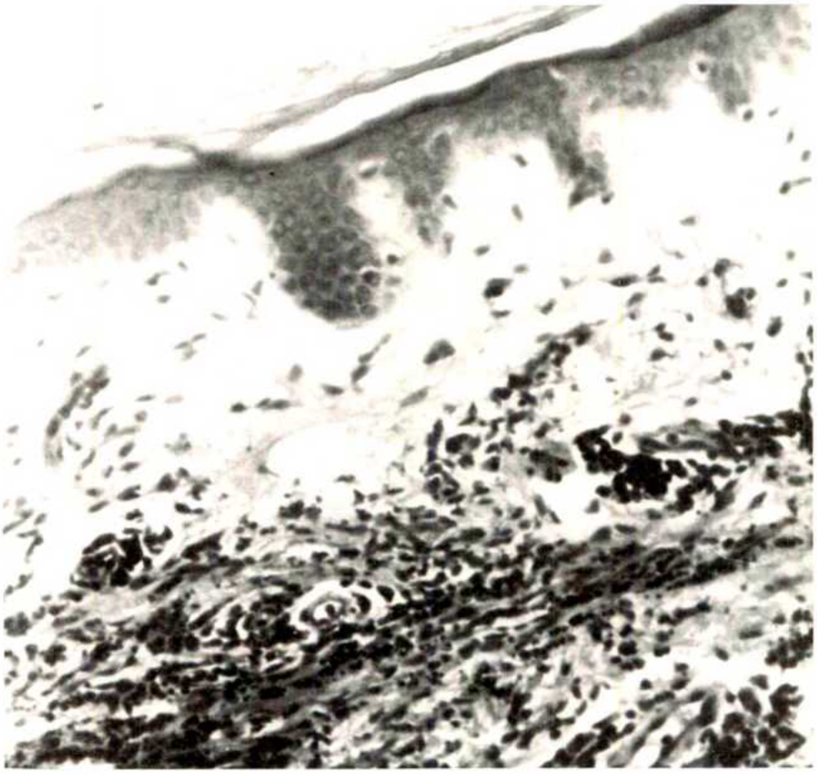

FIGURE 1. Photomicrograph from spontaneous skin lesion. Note the marked cellular infiltrate around the capillaries in the upper dermis with striking fragmentation of the nuclei producing 'nuclear dust' (H & E, × 750).

The patient was studied in detail in March 1974, when his skin lesions were widespread. Further spontaneous lesions were biopsied and compared with positive (Fig. 2) and negative skin tests induced by fractions of autologous serum. Further studies were carried out in July 1974, when the disease was in remission. The following laboratory tests were normal throughout:

Haemoglobin, white cell count, platelet count, plasma viscosity, ESR, protein electrophoresis, immunoglobulins, anti-streptolysin titre, urinalysis, creatinine clearance. X-rays of chest, hands and feet were normal.

Tests for circulating immune complexes are discussed in detail below. Immunofluorescent studies showed that IgG and complement were deposited in the spontaneous skin lesions. The patient was treated with oral prednisone in doses of between 20 and 60 mg per day, but the lesions continued to

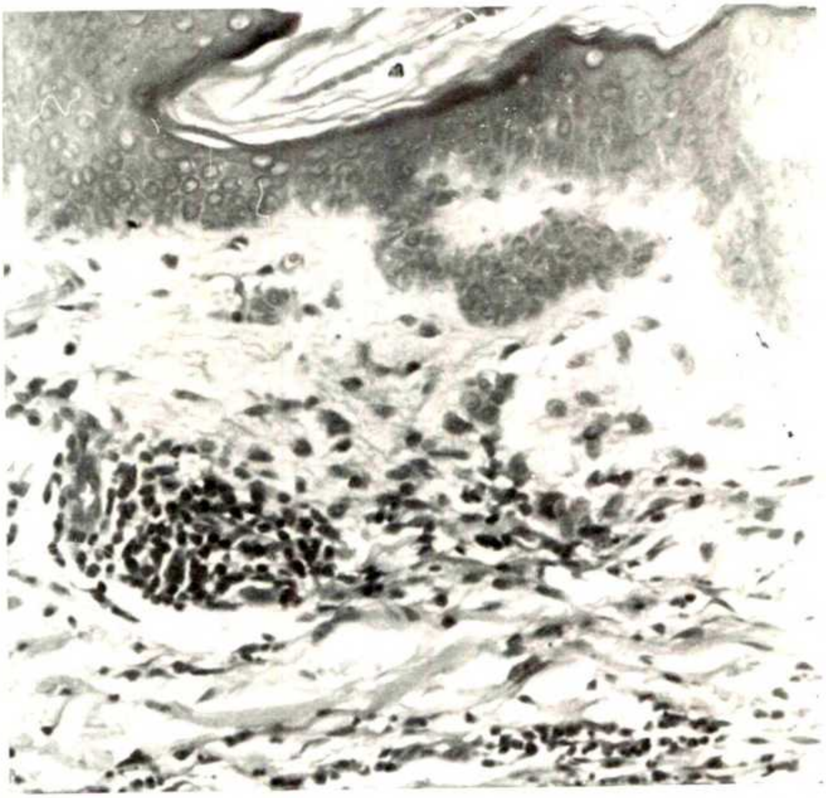

FIGURE 2. Photomicrograph from positive skin test showing essentially a similar picture to the spontaneous lesion (H & E, × 750).

recur. In March 1974 he was treated with an intensive course of plasmapheresis (which is currently under study in this department as a form of treatment for immune complex disease—Jones, Cumming & Asplin, in preparation). Following this his rash and general condition showed striking improvement and prednisone was discontinued. The improvement, however, proved to be temporary.

MATERIALS AND METHODS

C3 and C4 levels were determined by radial immunodiffusion using commercial plates (Hoechst). C3 activation products were detected by crossed immunoelectrophoresis using the method of Versey (1973). Anti-complementary activity was determined by inhibition of 2MHD of preserved guinea-pig complement at 37° C and 4° C in a system of sheep erythrocytes and horse haemolysin (Roitt & Doniach, 1966; Cream, 1973). Total haemolytic complement was measured in the same system. The ability of serum to cause platelet aggregation was measured by the method of Penttinen & Myllylä (1968). C1q was prepared from fresh normal serum and used in a precipitin reaction in agarose gel with test sera (Agnello, Winchester & Kunkel, 1970). Sera were also tested with a monoclonal rheumatoid factor giving a precipitin reaction with immune complexes (Winchester, Kunkel & Agnello, 1971). Serum from the patient was fractionated on a column of Sephadex G-200 in barbitone–HCl buffer (pH 8·6, ionic strength 0·1). Fractions were collected and assayed for IgG and IgM by radial immunodiffusion (Mancini, Carbonara & Heremans, 1965). For skin testing, selected fractions were dialysed against 0·15 M saline overnight at 4°C, and sterilized by membrane filtration. 100 μl of material to be tested was injected intradermally. Aliquots of the active fraction were treated with commercial antiserum to IgG and IgA overnight at 4°C with dialysis against 0·15 M saline. The precipitate was removed by centrifugation. The supernatant was sterilized and injected as before.

Histological and immunofluorescent techniques

Skin biopsies were divided. One portion was fixed in 10% buffered neutral formalin for routine histology while the other was snap frozen in liquid nitrogen for immunofluorescent studies. These were performed as follows: 5 μm frozen sections were cut on a Cryostat (SLEE). Sections were transferred to Teflon coated slides. The sections were dried in a stream of air for at least 1 h and then treated with fluorescein-labelled antisera to IgG, IgA, IgM (Wellcome), fibrin/fibrinogen and β_1C/β_1A globulin (Hoechst), using controls as described by Davies *et al.* (1973), and mounted in buffered glycerol at pH 7·3. The sections were examined and scored by two observers using a Wild M20 microscope under blue light fluorescence.

RESULTS

No evidence for circulating immune complexes was found. C3 and C4 levels were normal. No C3 activation products were found. CH50 levels were normal. No anti-complementary activity was detected at 37°C or 4°C. Platelet aggregation was within the normal range and no precipitation was detected with C1q or rheumatoid factor. The following tests were also negative:

WR, Hepatitis-B antigen, Coombs' test, fibrin degradation products in serum and urine, antinuclear factor, rheumatoid factor. Cold agglutinins were present at a titre of 1/8.

Skin testing

March 1974. Blood was collected under sterile conditions and 100 μl of fresh serum or fresh plasma was injected intradermally. There was no immediate reaction, but after 4 h, a nodular, erythematous lesion appeared at the site of injection, remained for 24 h, and then faded gradually over 2–3 days. Control injections of normal saline made at the same time were negative. The skin reactivity was lost from the serum and plasma on freezing.

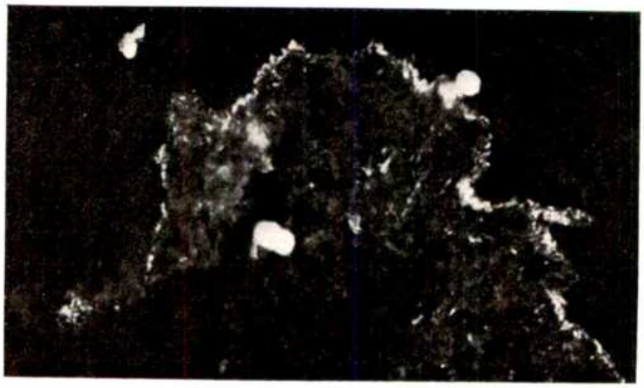

FIGURE 3. Immunofluorescent picture from the positive skin test showing the reaction to IgG. Note the heavy deposition in the basement membrane zone with a more diffuse weaker reaction around the capillaries in the upper dermis (× 375).

Fresh serum was fractionated on Sephadex. 0·5 ml of serum was applied to a column of Sephadex G-200 (Fig. 4), and the fractions were tested for skin reactivity. Activity was confined to a fraction between the main IgM and IgG peaks, and was abolished by treatment with anti-IgG antiserum, but not by anti-IgA. Fractions from this region of the curve were pooled and concentrated by dialysis against polyethylene glycol 6000, and chromatographed again on the G-200 column (Fig. 5). Two peaks of IgG were then seen, and skin reactivity was confined to the peak with higher molecular weight. Fractions from the concentrated, recycled material were negative when tested for anti-complementary activity.

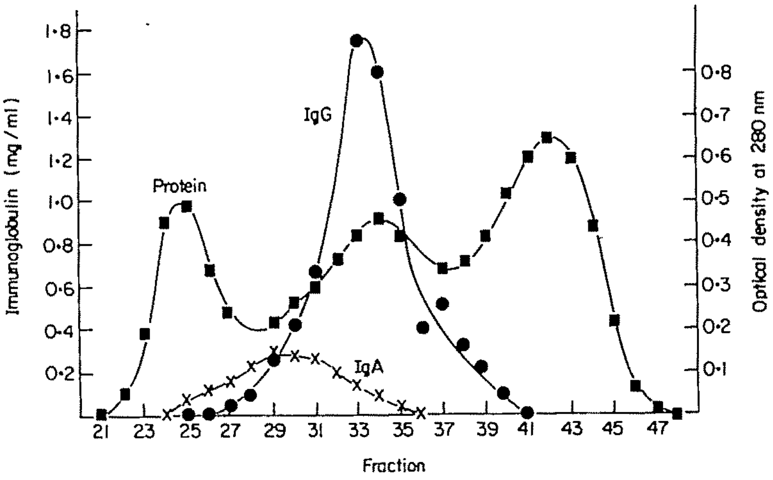

FIGURE 4. Fractionation of serum on G-200 Sephadex. Skin reactivity is limited to fraction 29, and is abolished by anti-IgG, but not by anti-IgA.
■——■ O.D. at 280 nm; ●——● IgG; ×——× IgA.

Skin test

Fraction	Reaction
24	–
29	+
29 reacted with anti IgA	+
29 reacted with anti IgG	–
31	–
33	–
Saline control	–

Immunofluorescent studies. Biopsies were taken from spontaneous skin lesions in March 1974, from lesions induced by the injection of active fractions of autologous serum, and from sites injected with non-active fractions. The results are summarized in Table 1. In the spontaneous lesions, IgG fluorescence was present in the epidermal basement membrane zone (BMZ) and in the walls of deep vessels. The positive skin test showed IgG deposition in the BMZ (Fig. 3), and more diffusely around the

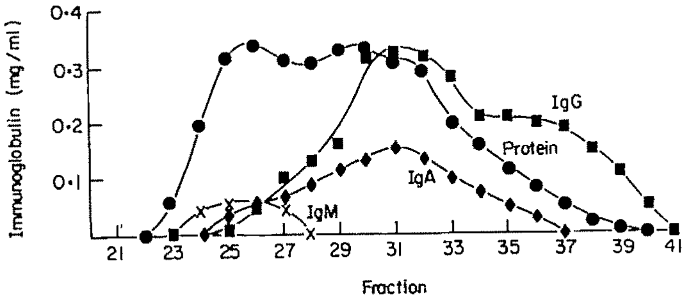

FIGURE 5. Results of recycling pooled fractions 26–30. Skin reactivity is now mainly in the higher molecular weight IgG peak.
●——● O.D. at 280 nm; ■——■ IgG; ◆——◆ IgA; ×——× IgM.

Skin test

Fraction	Reaction
27	Weakly +
29	+
31	+
33	+
35	−
Saline control	−

capillaries in the upper dermis. Complement was deposited within the small dermal vessels. The negative biopsy was from the site of injection of a non-active IgG fraction, and showed only a weak diffuse fluorescence with anti-IgG. No IgA or IgM was seen in any of the biopsies.

TABLE 1. Immunofluorescent studies of skin biopsies

	IgG	IgA	IgM	Complement
Biopsy of spontaneous skin lesion	++	−	−	−
Biopsy of positive skin test	++	−	−	+
Biopsy of negative skin test	+	−	−	−

July 1974. When the patient was in remission, with no rash, the injections of fresh whole autologous serum and plasma were repeated and produced no skin lesions. Material stored at −20°C from the previously positive fractions was also negative on skin testing; it is possible that activity had been destroyed by prolonged storage.

DISCUSSION

Christian (1960) showed that higher molecular weight heat-aggregated immunoglobulin would produce skin lesions on intradermal injection into normal subjects. Whitsed & Penny (1971) studied the vasculitis of a patient with cryoglobulinaemia, and found that autologous whole plasma and isolated cryoglobulin, injected intradermally, produced skin lesions similar to those occurring spontaneously.

Our observations provide strong evidence for the presence of an IgG component (presumably an immune complex) capable of initiating the skin lesions of acute necrotizing vasculitis. The immunofluorescent demonstration of IgG and complement in the positive skin test is further evidence that the lesions were caused by immune complex deposition. The histological appearances of the positive skin test and of the spontaneous lesion were strikingly similar, suggesting a common aetiology. The fact that complement deposition was only detected in the induced skin lesions may be related to the fact that they were biopsied at a very early stage of their evolution. It is of particular interest in the case we have described that all attempts to detect circulating immune complexes were unsuccessful. This failure may be due to the size of the complexes causing vasculitis and to the limitations of sensitivity of the methods employed. The active material detected in our system is clearly of low molecular weight (>7S, <19S). Precipitation with C1q occurs more readily with complexes of high molecular weight (Agnello *et al.*, 1970), and platelet aggregation is also induced by large (>19S) complexes (Hedfors & Norberg, 1974).

There is evidence that low molecular weight complexes are less readily removed from the circulation than those of higher molecular weight, which are taken up by the reticulo-endothelial system (Mannik & Arend, 1971), and this may explain the persistence of circulating complexes in necrotizing vasculitis. It is of interest that no evidence of complement consumption could be found in our patient although complement was detected by immunofluorescence in the positive skin test. It is possible that further aggregation of immune complexes may have taken place at the site of deposition, making them capable of fixing complement *in situ* (Lambert *et al.*, 1973).

In summary, we have described material corresponding to intermediate molecular weight IgG immune complexes which is capable of initiating typical skin lesions in a patient with necrotizing vasculitis. The observation, by establishing a direct biological assay for pathogenic immune complexes, offers new prospects for the further study of this group of diseases.

ACKNOWLEDGMENT

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REFERENCES

Agnello, V., Winchester, R.J. & Kunkel, H.G. (1970) Precipitin reactions of the C1q component of complement with aggregated γ-globulin and immune complexes in gell diffusion. *Immunology*, **19**, 909.

Christian, C.L. (1960) Studies on aggregated IgG. I. Sedimentation, electrophoretic and anticomplementary properties. *Journal of Immunology*, **84**, 112.

Cream, J.J. (1973) Anticomplementary sera in cutaneous vasculitis. *British Journal of Dermatology*, **89**, 555.

Davies, D.R., Tighe, J.R., Jones, M.F. & Brown, G.W. (1973) Recurrent haematuria and mesangial IgA deposition. *Journal of Clinical Pathology*, **26**, 672.

Hedfors, E. & Norberg, R. (1974) Evidence for circulating immune complexes in sarcoidosis. *Clinical and Experimental Immunology*, **16**, 493.

Jones, J.V., Cumming, R.H. & Asplin, C.M. (in preparation).

LAMBERT, P.H., BRICTEUX, N., SALMON, J. & MIESCHER, P.A. (1973) Dynamics of immune complex nephritis during antibody excess. *International Archives of Allergy and Applied Immunology*, **45**, 185.

MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235.

MANNIK, M. & AREND, W.P. (1971) Fate of preformed immune complexes in rabbits and Rhesus monkeys. *Journal of Experimental Medicine*, **134**, 19s.

MILNE, J.A. (1972) *An introduction to the diagnostic histopathology of the skin* p. 161. Arnold, London.

PARISH, W.E. (1970) Complexes of bacterial antigens with IgG or IgM antibodies in cutaneous vasculitis. *Proceedings of International Symposium on Immune Complex Diseases* (Ed. by L.Bonomo and J.L.Turk), p. 98. Carlo Erba Foundation, Milan.

PARISH, W.E. (1971) Studies on vasculitis. I. Immunoglobulins, β_{1C}, C-reactive protein and bacterial antigens in cutaneous vasculitis lesions. *Clinical Allergy*, **1**, 97.

PENTTINEN, K. & MYLLYLÄ, G. (1968) Interaction in human blood platelets, viruses and antibodies. I. Platelet aggregation test with microequipment. *Annales Medicinae Experimentalis et Biologiae Fenniae*, **46**, 188.

ROITT, I.M.R. & DONIACH, D. (1966) Complement-fixation test for thyroid, stomach, adrenal, mitochondrial and other non-organ specific cytoplasmic antibodies. In: *Serology Manual*, p. 13. World Health Organization, Geneva.

VERSEY, J.M.B. (1973) Automated two-dimensional immuno-electrophoresis and its application to the analysis of C3 and C4 in rheumatoid arthritis and systemic lupus erythematosus (SLE). *Annals of Clinical Biochemistry*, **10**, 100.

WHITSED, H.M. & PENNY, R. (1971) IgA/IgG cryoglobulinaemia with vasculitis. *Clinical and Experimental Immunology*, **9**, 183.

WINCHESTER, R.J., KUNKEL, H.G. & AGNELLO, V. (1971) Occurrence of γ-globulin complexes in serum and joint fluid of rheumatoid arthritis patients: use of monoclonal rheumatoid factors as reagents for their demonstration. *Journal of Experimental Medicine*, **134**, 286s.

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A comparison of histocompatibility antigens in dermatitis herpetiformis and adult coeliac disease

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SUMMARY

The incidence of histocompatibility antigens HL-A, 4a and 4b was studied in thirty-eight patients with dermatitis herpetiformis (DH) and thirty-six patients with adult coeliac disease (ACD). The 4b antigen was found in all the DH and ACD patients. HL-A 8 was found in 89% of patients with ACD—similar to the incidence reported in previous studies—and in 79% of patients with DH, a higher incidence than in previous studies which may be due to stricter criteria being used here to diagnose DH. There was no significant difference in the incidence of HL-A 8 between those patients with DH whose small intestinal biopsies appeared macroscopically abnormal and those with a normal macroscopic appearance. These findings suggest that patients with DH form a single disease group and do not support the concept previously postulated that there are two groups of patients with DH, one with an increased incidence of HL-A 8 antigen similar to that in ACD who have a gluten sensitive enteropathy (GSE), and another with a normal incidence of HL-A 8 antigen and without enteropathy.

The association between dermatitis herpetiformis (DH) and an enteropathy was first clearly demonstrated by Marks, Shuster & Watson (1966) and the enteropathy was soon shown to be gluten sensitive (Fry *et al.*, 1967, 1968). However, the incidence and significance of gluten sensitive enteropathy (GSE) in DH is still disputed. Gebhard *et al.* (1973) adhere to the earlier view that two-thirds of patients with DH have GSE and suggest that only in these patients is the incidence of HL-A 8 raised, as it is in adult coeliac disease (ACD) (Falchuk, Rogentine & Strober, 1972; Stokes *et al.*, 1972). However, Fry & Seah (1974) now believe that all patients with DH have GSE, and that earlier reports of only two-thirds of patients having GSE were based on inadequate criteria for diagnosing DH and GSE. There are also two different views held on the relationship between the skin and intestine in DH. First, there are those who believe that the relationship is direct and that both the skin and gut lesions are gluten dependent (Fry *et al.*, 1968, 1969, 1973; Barnetson, Heading & White, 1973). Secondly, there is the view that the relationship is indirect and the skin lesions are not gluten dependent (Shuster, Watson & Marks, 1968; Weinstein *et al.*, 1971; Gebhard *et al.*, 1973).

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There is now increasing evidence that immunological processes may be involved in the pathogenesis of both DH and ACD, and that the immunological disorder may be reflected by a high frequency of certain histocompatibility antigens. Using more precise criteria for the diagnosis of DH and GSE, we have studied the incidence of histocompatibility antigens, including for the first time, the complex of antigens 4a and 4b of van Rood in patients with DH and ACD, with particular reference to the presence and severity of the intestinal lesion in DH. The results of this study clearly demonstrate that DH patients fall into a single group and cannot be divided according to the presence of a macroscopic gut lesion and increased incidence of HL-A 8 on the one hand, and absence of both these factors on the other.

PATIENTS AND METHODS

Patients and controls

Thirty-eight patients with DH and thirty-six patients with ACD were investigated. The diagnosis of DH was made on clinical and histological features, the response of the skin lesions to dapsone and reappearance of the rash on withdrawal of the drug, and the presence of IgA in the uninvolved skin (Fry & Seah, 1974). The diagnosis of ACD was made in patients with a flat, or flat-with-mosaic proximal small bowel mucosal appearance (Holmes, Hourihane & Booth, 1961), and the characteristic histological features, particularly decreased cell height and increased lymphocytic infiltration of the small intestinal epithelium.

Controls were provided from the Tissue Typing Laboratory at St Mary's Hospital. Data from 180 individuals, mainly pregnant women, blood donors and laboratory staff were used, representing a cross-section of a London population, from which the patients were derived. For the 4a and 4b antigen, data from 923 similar controls were used. These controls were typed with the same anti 4a and anti 4b sera as the disease population.

Histocompatibility typing

Lymphocytes from defibrinated blood were used in a modified two-stage Kissmeyer-Neilsen lymphocyte micro-toxicity test. Antisera from multiple pregnancy and sensitized kidney transplant patients were used: a total of 171 antisera were employed, and the range of antisera to each of the histocompatibility antigens varied from 1:17 different sera. The specificities tested in the first and second segregant series were HL-A 1, 2, 3, 5, 7, 8, 9, 10, 11, 12, 13, 14, 17, 27, 28 and W5, W10, W15, W21, W22, W29, W32, Da25. The sera used for typing were a mixture of locally obtained sera and those obtained from a number of typing centres. The specificities have been validated by parallel typing with the National Tissue Typing Reference Laboratory and London Hospital plates. Patients were also tested for the histocompatibility antigens 4a and 4b of van Rood whose relationship to the HL-A antigens remains, at the present time, uncertain. It was decided to test for these antigens in view of the known frequent association of 4b with the HL-A 1, 8 chromosome. The 4a and 4b antisera were in part a gift from Professor J.J.van Rood and some local sera which consistently gave similar reactions to these.

Small intestinal biopsies

Small intestinal biopsies were taken before treatment with a gluten-free diet. The biopsies were taken from the duodeno-jejunal flexure under radiographic control, using a Crosby capsule and polythene guide cuff as described by Evans *et al.* (1970). Specimens were examined under a dissecting microscope for their macroscopic appearances and then processed routinely for histology. Quantification of intra-epithelial lymphocytic infiltration was also performed in patients with DH according to the method of Fry *et al.* (1972).

TABLE 1. Percent frequency of antigens detected in controls, dermatitis herpetiformis and adult coeliac disease patients

	HL-A															Workshop antigens							
	1	2	3	5	7	8	9	10	11	12	13	14	17	27	28	5	10	15	21	22	29	32	Da25
Controls N = 180	31·7	47·2	22·2	9·4	21·7	26·1	18·9	8·3	12·8	32·8	3·3	7·8	8·9	9·4	11·1	15·0	11·1	10·6	2·2	4·4	2·8	5·6	2·8
Coeliac disease N = 36	74·9	38·9	16·7	8·3	11·1	88·9	5·6	11·1	0	33·3	0	0	7·8	2·8	5·6	0	5·6	2·8	13·9	0	5·6	13·9	5·6
Dermatitis herpetiformis N = 38	60·5	41·0	23·0	5·0	17·9	78·9	20·9	0	10·3	38·1	0	0	0	3·2	6·4	11·3	6·1	16·8	3·2	11·6	0	16·0	0

RESULTS

Histocompatibility antigens

The frequency of detection of the HL-A antigens in control subjects and those with ACD and DH are shown in Table 1.

In the controls HL-A 1 was present in 32%, HL-A 8 in 26% and HL-A 1 and 8 existing together in 19%. In the patients, HL-A 1 was detected in twenty-three (61%) of the thirty-eight DH patients and in twenty-seven (75%) of the thirty-six ACD patients. HL-A 8 was detected in thirty (79%) of the DH patients, and in thirty-two (89%) of the ACD patients. HL-A 1 and 8 were found together in

TABLE 2. Tissue typing results in thirty-eight patients with dermatitis herpetiformis

	HL-A 1	HL-A 8	Others	4a	4b
1	+	+	3,12	+	+
2	−	+	2, 12	+	+
3	−	+	2, 12, W28	+	+
4	+	+	W18	−	+
5	−	−	2, 5, 12, W29	−	+
6	+	+	12, W29	+	+
7	+	−	3, 5, W5	+	+
8	−	+	2, 3, 7	−	+
9	+	+	12, W29	+	+
10	+	+	11, W22	−	+
11	−	−	2, 12, W29, W18	+	+
12	+	+	2, W5	−	+
13	+	+		−	+
14	+	+	2, W10	−	+
15	+	+	2, W27	+	+
16	−	−	2, W18, W27	+	+
17	+	+	3, W10	−	+
18	+	+	7	−	+
19	−	−	3, 12, W22, W29	+	+
20	+	+	11, W5	−	+
21	+	+	12, W29	+	+
22	+	+	3, 7	−	+
23	−	−	3, 7, 9, W18	+	+
24	+	+	3, 7	−	+
25	−	+	2, 9, 12	+	+
26	−	+	2, 9, W21	+	+
27	+	+	12, W29	+	+
28	−	−	9	+	+
29	+	+	2, 7	−	+
30	−	+	9, W32, W18	−	+
31	+	+	11, W18	−	+
32	+	+	9, W5	−	+
33	−	−	9, 10, 12, W18	+	+
34	+	+	12, W29	+	+
35	+	+	2, W15	−	+
36	−	+	12	+	+
37	−	+	7, 9	+	+
38	+	+	W18	−	+

twenty-two (58%) of the thirty-eight DH patients and in twenty-seven (75%) of the thirty-six ACD patients. The incidence of HL-A 1 was significantly greater than in the controls in both the DH ($P<0{\cdot}01$) and ACD ($P<0{\cdot}001$) groups, as were the incidences of HL-A 8 alone ($P<0{\cdot}001$) and of HL-A 1 and 8 together ($P<0{\cdot}001$). The deficit of other antigens in the patients accompanying the increase in HL-A 1 and HL-A 8 is widely distributed, and there is no significant deficit in the frequency of any particular antigen.

4a, 4b antigens

Of the 923 controls, 786 (85%) typed 4b positive, 337 (37%) had 4b alone, whilst 449 (49%) typed as 4a, 4b positive. In 137 (15%) 4a was found alone.

4b was found in all the thirty-eight DH and thirty-six ACD patients.

TABLE 3. Tissue typing results in thirty-six patients with adult coeliac disease

	HL-A 1	HL-A 8	Others	4a	4b
1	−	+	3, 7	−	+
2	+	+	2, W18	−	+
3	+	+	3, W18	−	+
4	+	+		+	+
5	+	+	W18	−	+
6	+	+	12	+	+
7	−	+	2, 7	−	+
8	+	+	2	−	+
9	+	+	12	+	+
10	−	−	10, 12, Da25	+	+
11	+	+	5	+	+
12	−	−	2, Da25, W17, W22	+	+
13	+	+	10	+	+
14	−	+	2, W10	−	+
15	+	+	3, W15	+	+
16	+	+	10, 12	+	+
17	−	+	2, 9, W18	−	+
18	+	+	2	−	+
19	+	+	2, W27	+	+
20	+	+	2, 12	+	+
21	+	+	Da25	+	+
22	+	+	2	+	+
23	+	+	12, W32	+	+
24	+	+		−	+
25	+	+	9, 12	+	+
26	+	+	3, 7	−	+
27	−	+	2, 3, 12	+	+
28	+	+	12, W29	+	+
29	−	−	Da25, W10, W18	−	+
30	+	+	12	+	+
31	+	+	10	+	+
32	+	+	5, Da25	+	+
33	+	+	2, W15	−	+
34	−	−	3, 5, 7, W32	+	+
35	+	+	2, 12	+	+
36	+	+	12, W29	+	+

The incidence of 4a antigen was not significantly different from the control population at the 5% level, but the differences in the incidence of 4b is greater than in controls in both DH and ACD ($P < 0{\cdot}05$). However, although 4b was present in all the patients, if a simple correction is made for the probability when twenty-five antigens are tested, the result no longer reaches significance at the 5% level (Walford, Smith & Waters, 1971).

The full results of the histocompatibility testing are given for the DH patients in Table 2, and for the ACD patients in Table 3.

Small intestinal findings

All the patients with ACD had, by definition, a flat or flat-with-mosaic proximal jejunal mucosa. Of the thirty-eight biopsies from the DH patients, sixteen (42%) had a normal (leaves or fingers and leaves) macroscopic appearance, whilst twenty-two (58%) were abnormal (flat or convoluted). Microscopically, however, fifteen of the sixteen small intestinal biopsies with a normal macroscopic appearance showed increased lymphocytic infiltration of the epithelium, as did twenty-one of the twenty-two biopsies with an abnormal macroscopic appearance. Thus, thirty-seven of the thirty-eight biopsies showed some abnormality.

Relation of HL-A antigens to small intestinal biopsy findings

In the two DH groups the incidence of HL-A 1 was 58% in the macroscopically normal biopsies, and 61% in the abnormal biopsies. The incidence of HL-A 8 was 88% in the normal macroscopic group and 63% in the abnormal macroscopic group (Table 4). HL-A 1 and 8 together was 53% in the normal group and 57% in the abnormal macroscopic group. There was no significant difference in the incidences of HL-A 1, HL-A 8 and HL-A 1 and 8 together between the two DH groups. Similarly, there was no difference between the incidence of HL-A 1 and HL-A 8 (Table 1) in the group of patients with DH and those with ACD.

TABLE 4. Relation of HL-A 8 positivity within each group

	DH		
	Small intestinal biopsy		
	Normal macroscopic appearance	Abnormal macroscopic appearance	ACD
Total	16	22	36
HL-A 8 positive	14 (88%)	15 (63%)	32 (88%)
HL-A 8 negative	2 (12%)	7 (37%)	4 (12%)

DISCUSSION

These results confirm that in both DH and ACD, the incidences of HL-A 1 and HL-A 8 separately, and of HL-A 1 and 8 together are raised. However, HL-A 1 and 8 are products of closely linked gene loci, and in north-western European populations there is a linkage disequilibrium resulting in the frequent inheritance of a chromosome HL-A 1, 8 (Albert, Mickey & Terasaki, 1971). Our results are consistent with the hypothesis that the increased incidence of HL-A 1 is due to the greater increase in HL-A 8, and is not an independent phenomenon. This finding is in agreement with Falchuk & Strober (1972) and thus it would appear that the raised incidence of HL-A 8 is the abnormality most likely to be associated with gluten sensitivity.

The present finding of an incidence of 79% for HL-A 8 in patients with DH is higher than in any previous study. Katz *et al.* (1972) reported an incidence of 58%, White *et al.* (1973) 60%, and Gebhard *et al.* (1973) 68%. The incidence of HL-A 8 in our control group was similar to that found in the previous studies, and thus the greater incidence of this antigen in our DH patients cannot be explained by a difference in the populations. It is more likely that the criteria adopted for diagnosing DH in the present study are more restricted. A small number of patients reported in the previous studies may not, in fact, have suffered from DH, within our definition, and hence have not shown the genetic association. Indeed, Fry & Seah (1974) have shown that if only the criteria of clinical and histological features, and response of the rash to dapsone are used for diagnosis as in the studies of Katz *et al.* (1972), White *et al.* (1973) and Gebhard *et al.* (1973), it is possible that up to 15% of patients may not have the disease. If the assumption is made that 15% of patients in these other studies did not have DH, the incidence of HL-A 8 would be increased to a similar incidence as reported here.

The incidence of 79% of HL-A 8 in patients with DH is not significantly different from 89%, the incidence of this antigen found here in ACD, and is similar to that reported by other workers in ACD —88% by Stokes *et al.* (1972) and 87% by Falchuk *et al.* (1972).

Recently, Gebhard *et al.* (1973) have suggested that there are two groups of patients with DH, one having GSE and showing a similar incidence of HL-A 8 (84%) to patients with ACD, and a second group who do not have GSE and who do not show an incidence of HL-A 8 greater than controls. Gebhard *et al.* (1973), however, divided their group of twenty-eight patients into nineteen who showed macroscopic and histological evidence of an enteropathy, and nine patients who did not show evidence of an enteropathy. The criteria they used to define an abnormal macroscopic or histological appearance were not given. Fry *et al.* (1974) have recently shown that in nearly half the patients with DH, the intestinal mucosa has a normal macroscopic appearance, but if quantification of the intra-epithelial lymphocytes is carried out, the incidence of abnormality of a single biopsy of the small intestine is found to be approximately 95%, and this is similar to the figure for abnormality found by Brow *et al.* (1971) using a multiple biopsy technique. In this present study, there was no significant difference in the incidence of HL-A 8 between the patients whose biopsies had an abnormal macroscopic appearance and those with a normal appearance (Table 4). It may be possible to reconcile the findings in the two studies if a small number of patients in the series by Gebhard *et al.* (1973) did not have DH. If one applies the figure of 15% for misdiagnosis mentioned above, it is possible that four of the twenty-eight patients in their study did not have the disorder. If this is so, these four patients would almost certainly belong to the group of nine patients in whom they found no enteropathy.

The concept of a significant group of patients with DH without an enteropathy is, indeed, now no longer tenable in view of the findings of Brow *et al.* (1971), Fry *et al.* (1972, 1976) and Weinstein (1973) which suggest that all patients with DH have an enteropathy. This present study, as in that of White *et al.* (1973), shows that irrespective of the severity of the intestinal lesion judged by morphological appearances, the incidence of HL-A 8 is similar.

A feature of the tissue typing in ACD and DH is the very high incidence of the 4b antigen which occurred in all thirty-six ACD and thirty-eight DH patients studied. It is not possible in this study to decide whether the association of the diseases with the 4b complex of antigenic specificities is better than that for HL-A 8.

It has now been shown conclusively that both the skin and intestinal lesions in DH are associated with gluten intake (Fry *et al.*, 1968, 1969, 1973; Barnetson *et al.*, 1973), and the finding of significantly raised incidences of HL-A 8 in DH and ACD reflects further evidence of a possible immunological abnormality in these disorders and a common aetiology in their pathogenesis.

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REFERENCES

ALBERT, E.D., MICKEY, M.R. & TERASAKI, P.L. (1971) Genetics of four new HL-A specifications in the caucasian and negro populations. *Transplantation Proceedings*, 3, 95.

BARNETSON, R.St.C., HEADING, R.C. & WHITE, A.G. (1973) HL-A 8 and dermatitis herpetiformis. *Lancet*, ii, 1027.

BROW, J.R., PARKER, F., WEINSTEIN, W.M. & RUBIN, C.E. (1971) The small intestinal mucosa in dermatitis herpetiformis. 1. Severity and distribution of the small intestinal lesion and associated malabsorption. *Gastroenterology*, 60, 355.

EVANS, N., FARROW, L.J., HARDING, A. & STEWART, J.S. (1970) New techniques for speeding small intestinal biopsy. *Gut*, 11, 88.

FALCHUK, Z.M., ROGENTINE, G.N. & STROBER, W. (1972) Predominance of histocompatibility antigen HL-A 8 in patients with gluten-sensitive enteropathy. *Journal of Clinical Investigation*, 51, 1602

FALCHUK, Z.M. & STROBER, W. (1972) HL-A antigens and adult coeliac disease. *Lancet*, ii, 1310.

FRY, L., KEIR, P., MCMINN, R.M.H., COWAN, J.D. & HOFFBRAND, A.V. (1967) Small intestinal structure and function and haematological changes in dermatitis herpetiformis. *Lancet*, ii, 729.

FRY, L., MCMINN, R.M.H.. COWAN, J.D. & HOFFBRAND, A.V. (1968) Effect of a gluten-free diet on dermatological, intestinal and haematological manifestations of dermatitis herpetiformis. *Lancet*, i, 557.

FRY, L., MCMINN, R.M.H., COWAN, J.D. & HOFFBRAND, A.V. (1969) Gluten-free diet and reintroduction of gluten in dermatitis herpetiformis. *Archives of Dermatology*, 100, 129.

FRY, L., SEAH, P.P., MCMINN, R.M.H. & HOFFBRAND, A.V. (1972) Lymphocytic infiltration of epithelium in diagnosis of gluten-sensitive enteropathy. *British Medical Journal*, iii, 371.

FRY, L., SEAH, P.P., RICHES, D.J. & HOFFBRAND, A.V. (1973) Clearance of skin lesions in dermatitis herpetiformis after gluten withdrawal. *Lancet*, i, 288.

FRY, L. & SEAH, P.P. (1974) Dermatitis herpetiformis: An evaluation of diagnostic criteria. *British Journal of Dermatology*, 90, 137.

FRY, L., SEAH, P.P., HARPER, P.G., HOFFBRAND, A.V. & MCMINN, R.M.H. (1976) The small intestine in dermatitis herpetiformis. In preparation.

GEBHARD, R.L., KATZ, S.I., MARKS, J., SHUSTER, S., TRAPANI, R.J., ROGENTINE, G.N. & STROBER, W. (1973) HL-A antigen type and small intestinal disease in dermatitis herpetiformis. *Lancet*, ii, 760.

HOLMES, R., HOURIHANE, O'B.D. & BOOTH, C.C. (1961) Dissecting microscope appearances of jejunal biopsy specimens from patients with 'idiopathic steatorrhoea'. *Lancet*, i, 81.

KATZ, S.I., FALCHUK, Z.M., DAHL, M.V., ROGENTINE, G.N. & STROBER, W. (1972) HL-A 8: A genetic link between dermatitis herpetiformis and gluten-sensitive enteropathy. *Journal of Clinical Investigation*, 51, 2977.

MARKS, J., SHUSTER, S. & WATSON, A.J. (1966) Small bowel changes in dermatitis herpetiformis. *Lancet*, ii, 128.

SHUSTER, S., WATSON, A.J. & MARKS, J. (1968) Coeliac syndrome in dermatitis herpetiformis. *Lancet*, i, 1101.

STOKES, P.J., ASQUITH, P., HOLMES, G.K.T., MACKINTOSH, P. & COOKE, W.T. (1972) Histocompatibility antigens associated with adult coeliac disease. *Lancet*, ii, 162.

WEINSTEIN, W.M., BROW, J.R., PARKER, F. & RUBIN, C.E. (1971) The small intestinal mucosa in dermatitis herpetiformis. II. Relationship of the small intestinal lesion to gluten. *Gastroenterology*, 60, 362.

WEINSTEIN, W.M. (1973) Latent coeliac sprue. *Gastroenterology*, 64, 819 (Abstract).

WHITE, A.G., BARNETSON, R.ST.C., DA COSTA, J.A.G. & MCCLELLAND, D.B.L. (1973) The incidence of HL-A antigens in dermatitis herpetiformis. *British Journal of Dermatology*, 89, 133.

WALFORD, R.L., SMITH, G.S. & WATERS, H. (1971) Histocompatibility systems and disease states with particular reference to cancer. *Transplantation Review*, 7, 78.

British Journal of Dermatology (1976) **94**, 139.

Histocompatibility antigens and dermatitis herpetiformis with special reference to jejunal abnormalities and acetylator phenotype

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SUMMARY

Sixty-one patients with dermatitis herpetiformis (DH) were studied. Of these fifty-three (87%) had the histocompatibility (HL-A) antigen HL-A8 as compared with 17% of a control population; the difference is significant. This incidence of HL-A8 among patients with DH is higher than that reported earlier for such patients and is similar to that reported for patients with adult coeliac disease. The incidence of HL-A1 was also significantly greater in the patient group, and was attributed to the increased frequency of haplotype HL-A1, 8. The frequency of HL-A8 was about equal in patients in whom DH was associated with jejunal abnormalities and in those in whom it was not. When correlated with rate of acetylation, there was no significant difference in the occurrence of various HL-A antigens.

The relationship of dermatitis herpetiformis (DH) to adult coeliac disease (ACD) is still only poorly understood. A common underlying factor may characterize these diseases since a high proportion of patients with DH have abnormalities of the jejunal mucosa similar to those typical of ACD (Marks, Shuster & Watson, 1966), and since these jejunal abnormalities in patients with DH also respond to a gluten-free diet (GFD) (Fry *et al.*, 1968). That the mutual factor may be a genetic one is suggested by results of recent studies on HL-A antigens which have shown that patients with ACD (Falchuk, Rogentine & Strober, 1972; Stokes *et al.*, 1972) and patients with DH (Katz *et al.*, 1972; White *et al.*, 1973) have a significantly higher incidence of HL-A8 than do control subjects. However, the frequency of HL-A8 has until now been reported to be lower in DH than in ACD. According to Gebhard *et al.* (1973), the frequency of HL-A8 in patients who have DH associated with jejunal abnormalities is similar to that in ACD, whereas in patients who have no jejunal abnormalities it is similar to that in healthy controls.

Previous studies have shown that individuals can be classified into two distinct phenotypes according to the rates at which they acetylate certain drugs. Dapsone, which is widely used to control the rash in DH, is acetylated by patients at various rates (Gelber *et al.*, 1971). Hence, patients with DH can be grouped into slow and rapid acetylators based on the rate at which they acetylate this drug.

In this study we have looked for various HL-A antigens in patients with DH, and have correlated the presence of these antigens with jejunal abnormalities and with the rate of acetylation.

PATIENTS

Sixty-one unrelated patients with DH (45 males and 16 females), whose mean age was 38 years, were studied. The mean duration of their disease was 6½ years. At the time of HL-A determinations twenty-seven patients were receiving dapsone, one sulphapyridine, twenty-five were on a GFD and receiving dapsone and eight were on a GFD alone. The diagnosis of DH was based on a typical clinical picture of the rash and fulfilment of at least three of the following criteria: (1) clearance of the rash with dapsone; (2) relapse of the rash after withdrawal of dapsone; (3) histological demonstration of subepidermal blistering and the presence of papillary microabscesses around the blisters; and (4) demonstration of IgA in the skin.

METHODS

HL-A antigens were identified by the microlymphocytotoxicity test (Amos *et al.*, 1969). For each specificity at least two reagent sera were employed, in each case always of both foreign and local origin. The control groups comprised one series of 326 randomly selected healthy Finns (Tiilikainen *et al.*, 1972) and another series of 120 employees of the blood bank and 280 blood donors.

Before introducing a GFD, a jejunal biopsy was performed under X-ray control with a Bults' biopsy instrument positioned immediately distal to the ligament of Treiz. The specimens obtained were fixed in formaldehyde, stained with haematoxylin and eosin, and classified histologically as mucosa that was normal; that containing increased amounts of lymphocytes and plasma cells without villous atrophy; or that showing partial, subtotal or total villous atrophy. Only findings of partial, subtotal or total villous atrophy were considered as clearly abnormal.

The acetylation capacity of fifty of the sixty-one patients studied was determined as described earlier (Förström, Mattila & Mustakallio, 1974). Immuno-histological examination of the skin biopsies taken from fifty-six patients, mostly from their lesions, was performed by direct immunofluorescence as described elsewhere (Salo, 1970). When specimens were stained with anti-human IgA conjugate (Behringwerke, batch no 256F) diluted 1:20, IgA was demonstrated in the dermal papillae and/or in the dermo-epidermal junction in the biopsies of fifty of these patients.

RESULTS

The frequencies of twenty-four HL-A antigens tested in control subjects and in patients with DH are given in Table 1. The incidence of HL-A1 and of HL-A8 was significantly ($P<0{\cdot}001$) higher among the patients than among the controls. HL-A1 was never detected in an HL-A8 negative individual. On the basis of frequencies of HL-A phenotypes and HL-A1, nonHL-A8 haplotypes demonstrated in the Finnish control populations, this series would have been expected to contain four HL-A1, non-HL-A8 phenotypes. The incidence of HL-A2 and W15 was in fact markedly lower, but if their occurrence is evaluated as a proportion of 'nonHL-A1' and 'nonHL-A8', respectively, the deviations are not significant.

Of the fifty-one patients on whom jejunal biopsy was performed thirty-nine (76%) were abnormal. Thirty-five (90%) of these thirty-nine patients had HL-A8. Of the twelve patients in whom the jejunal biopsy was normal this antigen was present in ten (83%). Hence the frequency of HL-A8 did not

TABLE 1. The incidence of HL-A antigens in healthy controls and in patients with dermatitis herpetiformis

Antigen	Controls (n = 326)		Dermatitis herpetiformis (n = 61)		Chi-square > 2 (Yates' corrected)
	No.	%	No.	%	
HL-A 1	55	17	39	64	59·36†
HL-A 2	180	55	21	34	8·08
HL-A 3	137	42	21	34	—
HL-A 9	60	18	5	8	3·14
HL-A 10	32	10	4	7	—
HL-A 11	34	10	3	5	—
HL-A 28	39	12	8	13	—
W 19	51	16	12	20	—
HL-A 5	41	13	4	7	—
HL-A 7	95	29	12	20	—
HL-A 8	57	17	53	87	118·26†
HL-A 12	48	15	6	10	—
HL-A 13	25	8	6	10	—
HL-A 14	0	0	0	0	—
HL-A 17	12	4	0	0	—
HL-A 27	46	14	5	8	—
W 5	61	19	11	18	—
W 10	50	15	3	5	3·88
W 15	84	26	7	11	5·07
W 16	39	12	5	8	—
W 18	16	5	3	5	—
W 21	0	0	1	2	—
W 22	23	7	0	0	—
TY	4*	1	0	0	—

* Tested in 120 blood bank employees and 280 blood donors.

† Highly significant *P*-value even after multiplication by 24, the number of HL-A specificities under study.

differ significantly in patients with normal and with abnormal biopsies. HL-A1 was present in these groups in 67% and 42%, respectively. When correlated with rate of acetylation, there was no significant difference in the occurrence of various HL-A antigens. HL-A8 occurred in 89% of twenty-eight slow and in 77% of twenty-two rapid acetylators. HL-A1 occurred in 68% and 50% of these groups respectively.

DISCUSSION

The incidence of the histocompatibility antigens HL-A1 and HL-A8 was significantly greater ($P < 0{\cdot}001$) in the present series of sixty-one patients with DH than in the control population. Furthermore, whereas in the patient population HL-A1 occurred only in the carriers of HL-A8, in the control population 35% of HL-A1 carriers lacked HL-A8. This corroborates the observation reported previously (Katz *et al.*, 1972) that an increase in the frequency of HL-A1 is attributable to the increase in that of HL-A8. The incidence of HL-A8 among patients with DH was 87%, as compared with 17%

among control subjects, and exceeded incidences published earlier for such patients. In three previously reported series of twenty-six, thirty-five and twenty-eight patients each, the frequencies of HL-A8 were 58%, 60% and 68% respectively (Katz *et al.*, 1972; White *et al.*, 1973; Gebhard *et al.*, 1973). The difference in this incidence between the present and earlier reports cannot be attributed to a more frequent occurrence of HL-A8 in the Finnish gene pool, since the control value (17%) was one of the lowest of the reports dealing with HL-A8 in DH and ACD.

There are patients in whom clinical picture, histological findings and response to dapsone resemble those in DH and yet, follow-up study shows that they do not have DH (Fry & Seah, 1974). The importance of the demonstration of IgA in the skin of such patients as the foremost diagnostic criterion of DH has already been pointed out (Seah & Fry, 1975). Only the most rigorous criteria were used in the diagnosis of DH in the present study, and this may be one reason for the uncommonly high incidence of HL-A8 in our report.

The incidence of HL-A8 in this study is similar to that published for patients with ACD, an illness in which it reportedly varies between 79 and 88% (Falchuk *et al.*, 1972; Stokes *et al.*, 1972, 1973; Strober *et al.*, 1974). An increased occurrence of HL-A8 has also been found in patients with childhood coeliac disease (McNeish, Nelson & Mackintosh, 1973; Granditsch *et al.*, 1973). Exacerbation by gluten is a mutual feature of these three diseases, all of which are characterized by jejunal abnormalities and by an increased frequency of HL-A8 which, moreover, seems to be related to the production of a high-titred anti-gluten antibody (Scott *et al.*, 1974). Furthermore, in most patients with DH the rash responds to a GFD (Fry *et al.*, 1973), and there is now considerable evidence to suggest that DH is due to an immunological abnormality associated with gluten sensitivity (Seah & Fry, 1975). Gebhard *et al.* (1973) observed that only those patients with DH who had jejunal abnormalities had a significant increase in the incidence of HL-A8 and that this incidence was equal to that found in ACD. They postulated, moreover, that HL-A8 is related independently to the skin disease and to the disease of the small-intestine. As did White *et al.* (1973), we also found no significant difference between the occurrence of HL-A8 in patients with and without jejunal manifestations. Hence, as pointed out by Barnetson, Heading & White (1973), it may not be valid to classify patients with DH into two groups on the basis of jejunal abnormalities determined from a single biopsy, because the distribution of abnormalities is often patchy and they exist in almost every patient. We regard the uncommonly high incidence of HL-A8 among the patients in this study as further evidence of a similarity between DH and ACD.

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REFERENCES

AMOS, D.B., BASHIR, H., BOYLE, W., MACQUEEN, M. & TIILIKAINEN, A. (1969) A simple microcytotoxicity test. *Transplantation*, 7, 220.

BARNETSON, R.ST.C., HEADING, R.C. & WHITE, A.G. (1973) HL-A8 and dermatitis herpetiformis. *Lancet*, ii, 1027.

FALCHUK, Z.M., ROGENTINE, G.N. & STROBER, W. (1972) Predominance of histocompatibility antigen HL-A8 in patients with gluten-sensitive enteropathy. *Journal of Clinical Investigation*, 51, 1602.

FRY, L., MCMINN, R.M.H., COWAN, J.D. & HOFFBRAND, A.V. (1968) Effect of gluten-free diet on dermatological, intestinal, and haematological manifestations of dermatitis herpetiformis. *Lancet*, i, 557.

FRY, L., SEAH, P.P., RICHES, D.J. & HOFFBRAND, A.V. (1973) Clearance of skin lesions in dermatitis herpetiformis after gluten withdrawal. *Lancet*, i, 288.

FRY, L. & SEAH, P.P. (1974) Dermatitis herpetiformis: an evaluation of diagnostic criteria. *British Journal of Dermatology*, **90**, 137.

FÖRSTRÖM, L., MATTILA, M.J. & MUSTAKALLIO, K.K. (1974) Acetylator phenotype, minimal maintenance dose and haemolytic effect of dapsone in dermatitis herpetiformis. *Annals of Clinical Research*, **6**, 308.

GEBHARD, R.L., KATZ. S.I., MARKS, J., SHUSTER, S., TRAPANI, R.J., ROGENTINE, G.N. & STROBER, W. (1973) HL-A antigen type and small-intestinal disease in dermatitis herpetiformis. *Lancet*, **ii**, 760.

GELBER, R., PETERS, J.H., GORDON, G.R., GLAZKO, A.J. & LEVY, L. (1971) The polymorphic acetylation of dapsone in man. *Clinical Pharmacology and Therapeutics*, **12**, 225.

GRANDITSCH, G., LUDWIG, H., POLYMENIDIS, Z. & WICK, G. (1973) Coeliac disease and HL-A8. *Lancet*, **ii**, 908.

KATZ, S.I., FALCHUK, Z.M., DAHL, M.V., ROGENTINE, G.N. & STROBER, W. (1972) HL-A8: a genetic link between dermatitis herpetiformis and gluten-sensitive enteropathy. *Journal of Clinical Investigation*, **51**, 2977.

MARKS, J., SHUSTER, S. & WATSON, A.J. (1966) Small-bowel changes in dermatitis herpetiformis. *Lancet*, **ii**, 1280.

MCNEISH, A.S., NELSON, R. & MACKINTOSH, P. (1973) HL-A1 and 8 in childhood coeliac disease. *Lancet*, **i**, 668.

SALO, O.P. (1970) SLE-like deposition of immunoglobulins in the skin in rosacea. *Annals of Clinical Research*, **2**, 28.

SEAH, P.P. & FRY, L. (1975) Immunoglobulins in the skin in dermatitis herpetiformis and their relevance in diagnosis. *British Journal of Dermatology*, **92**, 157.

SCOTT, B.B., SWINBURNE, M.L., RAJAH, S.M. & LOSOWSKY, M.S. (1974) HL-A8 and the immune response to gluten. *Lancet*, **ii**, 374.

STOKES, P.L., ASQUITH, P., HOLMES, G.K.T., MACKINTOSH, P. & COOKE, W.T. (1972) Histocompatibility antigens associated with adult coeliac disease. *Lancet*, **ii**, 162.

STOKES, P.L., ASQUITH, P., HOLMES, G.K.T., MACKINTOSH, P. & COOKE, W.T. (1973) Inheritance and influence of histocompatibility (HL-A) antigens in adult coeliac disease. *Gut*, **14**, 627.

STROBER, W., FALCHUK, Z.M., NELSON, D.L. & GEBHARD, R.M. (1974) The pathogenesis of gluten-sensitive enteropathy. In: *Progress in Immunology II*, Vol. 4, p. 221. North-Holland Publishing Company, New York.

TIILIKAINEN, A., ERIKSSON, A.W., MACQUEEN, J.M. & AMOS, D.B. (1972) The HL-A system in the Skolt Lapp population. In: *Histocompatibility testing*, p. 85. Munksgaard, Copenhagen, Denmark 1973.

WHITE, A.G., BARNETSON, R.ST.C., DA COSTA, J.A.G. & MCCLELLAND, D.B.L. (1973) The incidence of HL-A antigens in dermatitis herpetiformis. *British Journal of Dermatology*, **89**, 133.

British Journal of Dermatology (1976) 94, 145.

The action of a urea–lactic acid ointment in ichthyosis

WITH PARTICULAR REFERENCE TO THE THICKNESS OF THE HORNY LAYER

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SUMMARY

The effect of urea–lactic acid ointment on the scales of ichthyotic skin was assessed using the 'cell swelling' technique of Christophers & Kligman. The thickness of the horny layer and of the scales was reduced both by the ointment and by the base. The formation of scales was shown to differ from that in psoriasis where the scale forms at the apex of a rete peg and is symmetrical whereas in ichthyosis the separation of the scale may be at an entirely different depth at one margin. There is an increase in the number of 'T' cells in X-linked recessive ichthyosis.

Christophers & Kligman (1964) showed that it is possible, by means of a simple cell-swelling technique, to count the cell layers of the stratum corneum in health. Apart from investigations in acne vulgaris (Blair, 1968) and in the skin of old age (Baker & Blair, 1968), there has been no published work on the application of this method to pathological skin. This paper reports an attempt to assess the effects of a urea–lactic acid preparation in ichthyotic skin employing this technique.

Previous work by Swanbeck (1968) showed that urea may improve ichthyosis clinically and indicated that it might increase the uptake of water by the horny layer. Further work by Swanbeck (1968) using a urea–lactic acid cream (Calmurid, Pharmacia Ltd.) showed that there was a significant clinical response to the preparation in ichthyosis. We hoped that, by testing this cream in ichthyosis, we might be able to throw some light on the nature of the action of the substances on the scaling process.

MATERIALS AND METHODS

Patients suffering from autosomal dominant and X-linked recessive ichthyosis, who had attended Guy's Hospital, St John's Hospital for Diseases of the Skin or the London Hospital, were invited to take part in a clinical trial and consent was obtained for punch biopsies before and after treatment.

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The same patients were tested by Dr K. Grice and her colleagues for transepidermal water loss and water holding capacity before and after treatment (Grice, Sattar & Baker, 1973). All previous treatments were discontinued 21 days before the initial assessment and biopsy. The clinical assessment was carried out by Dr Grice and the patient's own assessment was also recorded.

Three preparations were used, Calmurid as marketed, Calmurid base (i.e. with urea, betaine and lactic acid omitted) and vitamin A acid 0·025% in yellow soft paraffin. These were used in coded containers and the patients were instructed to use the Calmurid cream to one upper limb and the base to the opposite upper limb.

The preparations were applied twice daily. The vitamin A acid ointment was applied to one thigh under occlusion. The biopsies were repeated after 2 weeks' treatment. Three millimetre punch biopsies were obtained from one arm and one thigh of each patient using a Keyes rotary manual skin trephine. The skins were not swabbed.

The sites selected were the extensor aspect of the upper arm and the outer aspect of the mid-thigh. An attempt was made to include a scaly area, visible to the naked eye, in each patient. Four to 6 μm cryostat sections were made and four out of every five sections were discarded. Three to four sections were mounted on each slide. In all, eight to ten slides were obtained from each biopsy specimen. Of these, two were fixed in formol saline and stained with haematoxylin and eosin and the remaining sections were stored in the deep freeze.

Sections were swollen with M/2 sodium hydroxide solution (Christophers & Kligman, 1964; Blair, 1968). They were examined using a $\frac{2}{3}$ objective and then a $\frac{1}{12}$ oil immersion objective. The cell layers were counted with a tally counter to eliminate bias.

RESULTS

Of eight cases treated with base, only five showed significant reduction in the number of horny layers. After treatment with Calmurid, a significant fall in horny cell layers occurred in six of eleven cases. An attempt was made to correlate clinical findings with horny layer thickness and with water-holding capacity. Water-holding capacity was enhanced in all cases by the urea cream and to a lesser extent by the base. There was no correlation with effect on horny layer thickness. Clinical improvement and reduction in horny layer thickness did correlate in all cases in which there was a significant drop in cell count (six cases). This fall happened in the most severe cases and in those in whom there was a noticeable clinical improvement there was also marked reduction in the thickness of the scales. The post-treatment findings are given in Table 1. Grice *et al.* (1973) found an increased water-holding capacity of the stratum corneum by 100% in all their cases treated with the urea cream. Transepidermal water loss was only slightly reduced. It was not possible to correlate their findings with the effect on the number of horny cells in this small series.

Effects on scaling

Occasionally thin sections resulted in tearing off of portions of the horny layer. However, the large number of sections made overcame this difficulty and although breaks occurred in the horny layer, it was possible to identify artefacts (Fig. 1).

Scales could be seen in most sections and the whole of the horny cells involved could be seen clearly in contrast with those which were artificially broken (Fig. 1).

In pre-treatment sections, scales consisted of several cell layers from two to twenty cells (Fig. 2). For non-scaling stratum corneum, the number of cells on the area adjacent to the free edge of the scale was equal to the number at the attached edge minus the number of cells constituting the scale.

TABLE 1. The effect of Calmurid cream and Calmurid base on the number of horny cell layers in ichthyotic skin

Case no.	Age	Sex	Autosomal dominant (AD) or sex-linked (SL)	Pre-treatment No. of cell layers	Post-treatment No. of cell* layers		Physician's assessment†	Patient's assessment
2	17	M	SL	50·2	C 13·6	0·001	++	++
3	18	M	SL	36·2	C 35·4	NS	++	++
					B 38·2	NS	o	o
4	18	F	AD	18·4	C 19·2	NS	++	++
8	53	M	AD	27·2	C 13·4	0·01	+	+
					B 12·0	0·01	o	o
9	55	F	AD	16·4	C 14·2	NS	++	++
					B 13·2	NS	++	++
10	27	M	SL	43·0	C 70·8	0·001	+	+
					B 22·0	0·01	o	o
11	15	F	AD	12·2	C 10·0	NS	+	++
					B 11·4	NS		
13	48	M	SL	27·0	C 19·0	0·1	++	++
					B 17·8	0·1	o	o
14	14	M	SL	27·2	C 17·8	0·05	++	++
					B 19·8	0·1	+	+
12	56	M	SL	26·2	C 15·4	0·02	+	++
					B 29·6	NS	+	+
15	70	F	AD	55·8	C 39·6	0·01	+	+

* C = urea; B = base.

† ++ = great improvement; + = improved; o = no change.

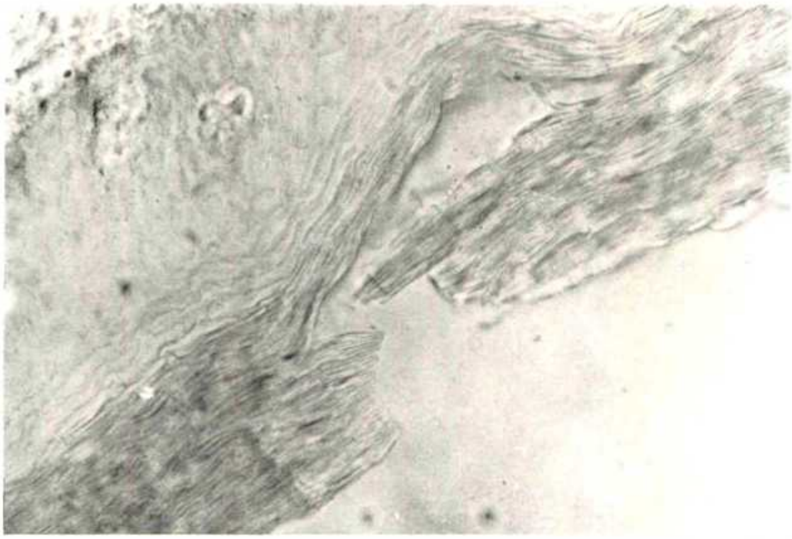

FIGURE 1. Skin from untreated thigh of patient with autosomal dominant ichthyosis sectioned in cryostat and swollen with M/2 NaOH, showing deeper layers of stratum corneum which have stretched and superficial layers, broken and retracted—plainly an artefact due to damage during sectioning.

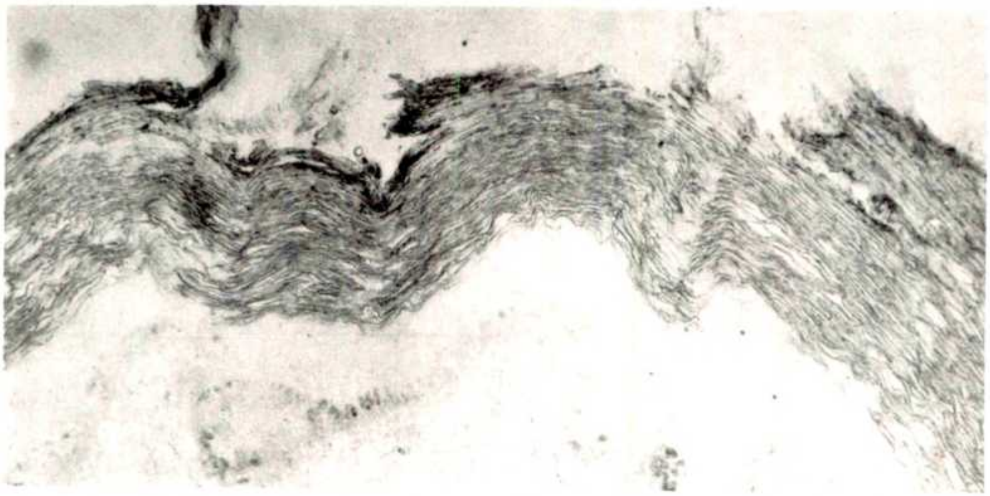

FIGURE 2. Skin from untreated arm of subject with X-linked ichthyosis, cut in cryostat and swollen with M/2 NaOH showing scales with 'frayed ends', which are thicker on one side than the other. Presumably the ground substance has been lost at different levels in different parts of the scale and therefore the scale will not separate until the ground substance is lost at the level corresponding with the deeper edge of the scale.

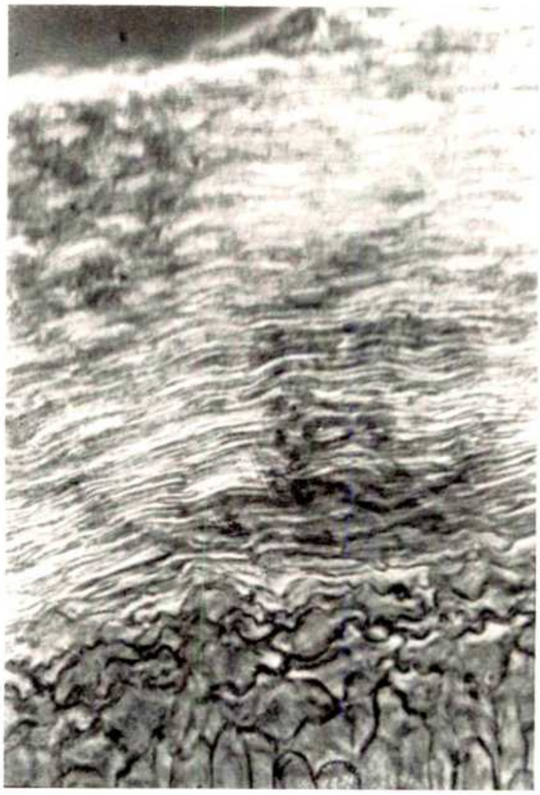

FIGURE 3. Skin from untreated thigh of patient with X-linked ichthyosis showing 4 layers of 'T' cells, treated as previous section, plus phase contrast.

Ghost cells

Thin-walled cells lying in the deepest layers of the stratum corneum have been noted (Blair, 1968) and are probably comparable with Brody's 'T' cells (Brody, 1959). In normal skin there are one to two layers of these. However, in X-linked recessive ichthyotic skin up to four layers of these were seen (Fig. 3).

Palisading

This feature was noted by Christophers (personal communication, 1966) and it was present in early samples taken from acne subjects. Mackenzie (personal communications, 1969 and 1971) has noted this feature in monkey skin and has shown that by stretching human skin samples it is a constant feature.

The author has not been able to stretch her 3 mm samples but, by means of pre-biopsy freezing with Arcton or with liquid nitrogen, less wrinkling of the specimens occurs and the palisading is accentuated and it is retained in X-linked recessive ichthyosis (Figs 4a and b). The persistence of

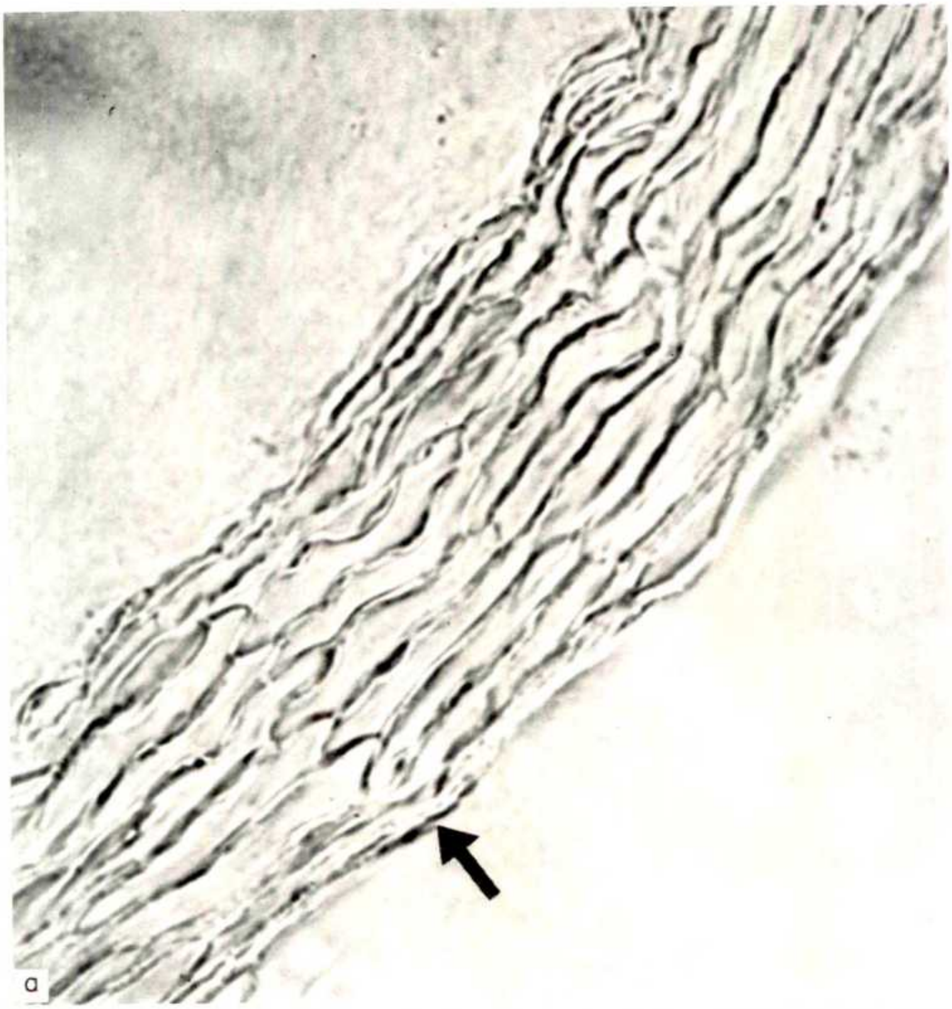

FIGURE 4(a). Skin from back of young adult treated as previously showing 'palisading or stacking'.

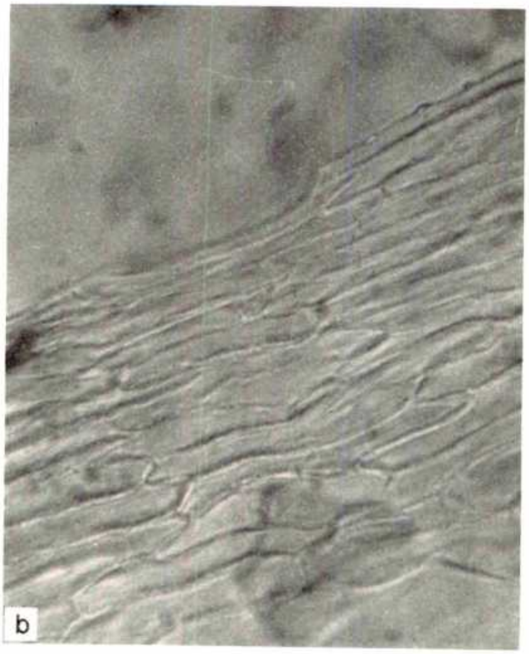

FIGURE 4(b). Skin from patient with X-linked recessive ichthyosis shows palisading.

palisading and the manner of formation of scales contrasted with psoriatic skin. In psoriatic skin the scales appear to be symmetrical and separation occurs circumferentially. The centre of the scale appears to correspond with the base of a dermal papilla and the thickness of the scale appears to be the same at all points on the periphery (Fig. 5). In ichthyotic skin there seems to be no relation between the scales and the papillae and no symmetry, so that in section, one edge of the scale may be a few cells thick and on the opposite edge of the section may be up to twenty or thirty cells thick. In one patient, granular material appeared between the horny cells and interfered with the counting technique.

DISCUSSION

The value of urea and of lactic acid as softening agents for keratin has been known for many years (Marchionini, 1934; Stern, 1946; Fabre-Teste, 1958; Armuzzi, 1962; Lageholn & Lodn, 1966).

This study shows that both the ointment, which contains urea and lactic acid, and the base without urea, lactic acid or betaine, were effective in reducing the thickness of the scales. It would be of interest to study the two agents separately.

The factors leading to normal scaling of the epidermis are far from understood. The arrival of new cells at the surface and the loss of one or more layers are minutely balanced and it is when this balance is lost that hyperkeratosis occurs. In psoriasis it is regularly found that the rate of replication of epidermal cells is speeded up and those arriving at the surface are immature. It is likely that the intercellular substance in these cases is also abnormal but because the degree of immaturity of the cells is approximately the same, they desquamate at the same time and this means that the scales are symmetrical. In contrast, in ageing skin the rate of replication is reduced and turnover time is prolonged (Baker & Blair, 1968). Here again one would expect the intercellular substance to be abnormal and it is found that the horny layer may be grossly increased in thickness. Scaling is usually scarcely perceptible and scales are thin (Blair, unpublished).

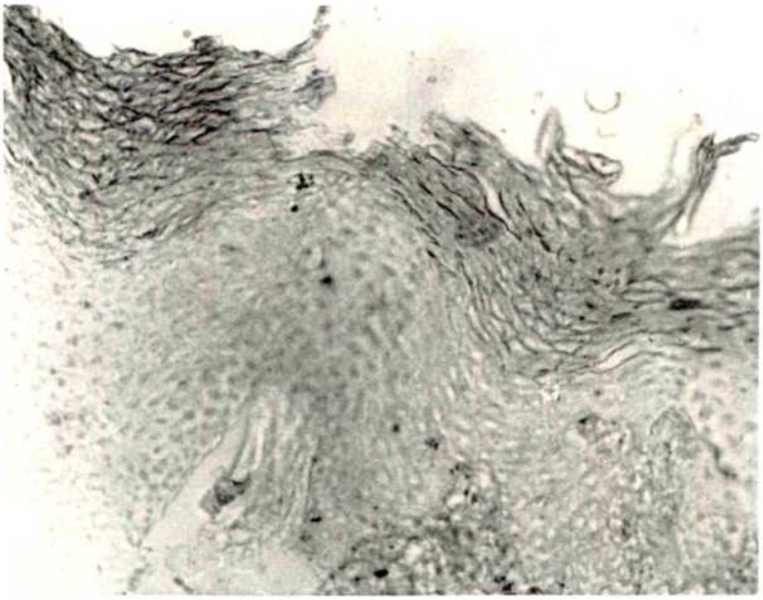

FIGURE 5. Section of psoriasis skin shows symmetrical scales each 'tethered' opposite rete peg.

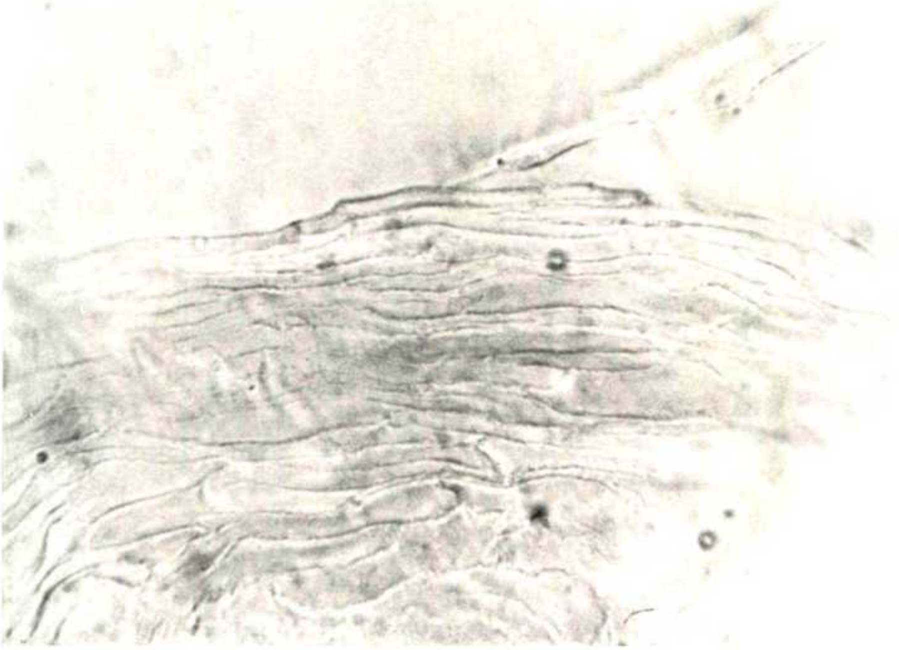

FIGURE 6. Skin from arm of patient with X-linked recessive ichthyosis treated with Calmurid, showing thin scales and horny layer of normal thickness, between scales.

In both autosomal dominant and X-linked recessive ichthyosis little is known about the cause of the scaling. The granular layer is poorly developed in the autosomal dominant type (Wells & Kerr, 1966; Feinstein, Ackerman & Ziprkowski, 1970) so that an abrupt transition from spinous cells to intermediate and transitional cells occurs. It is interesting, therefore, that in these swelled preparations there appears to be an increased number of 'T' cells, at any rate in the X-linked type, which in these preparations show elaborate interlockings of the adjacent cell walls. This may, of course, be an artefact. However, if the normal loss of horny cells depends upon degradation of the intercellular substance one would expect the increased amount of mucopolysaccharide to increase the water barrier properties of the skin as it does in the hippopotamus (Luck & Wright, 1964). Feinstein *et al.* (1970) noted that in both dominant and X-linked ichthyosis, minimal changes might be found in the skin of the arm in contrast to the thigh where scaling was greatest. The present study utilized skin mainly from the arm and it is possible, therefore, that the changes noted in X-linked ichthyosis may also occur in the dominant type had the study been extended to include more material from the more scaly lower limbs. The skin in ichthyosis is a poor barrier and retains water badly. On the other hand the defect in ichthyosis may lie in the quality of the horny cells themselves.

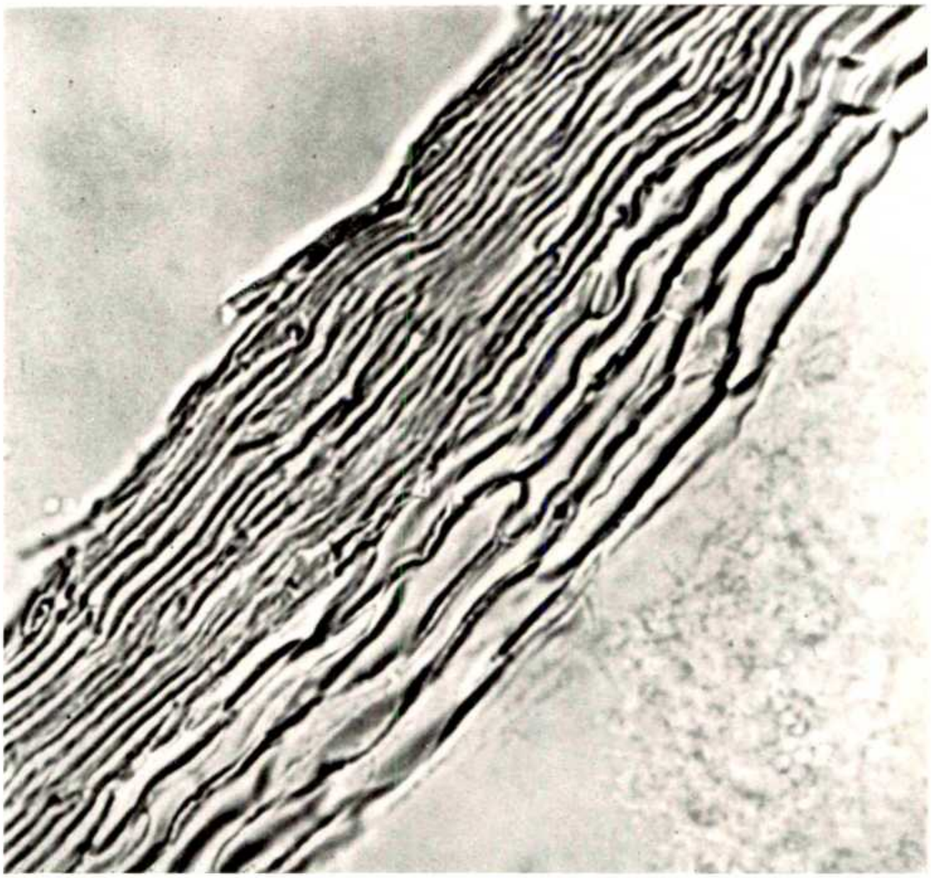

FIGURE 7. Unicellular scales separating caudally in section of normal skin.

Finally, the scales in swelled preparations show a large number of cell layers in one part of the section but there is no corresponding separation at a symmetrical position. This suggests that the condition or age of the intercellular substance is not the same at similar levels of the epidermis. The fact that palisading is retained in dominant ichthyosis suggests that the orderly arrangement demonstrated by Mackenzie, in which the horny cells are stacked in a hexagonal fashion in a fixed relationship to the Langerhans cells, is retained in autosomal dominant ichthyosis but that the fault probably does lie in the irregular maturation of the intercellular cement. In any event, the investigation has served to demonstrate some morphological abnormalities in ichthyosis and it substantiates the clinical impression of improvement.

Fig. 7 shows that normal horny cells and the unicellular scales are separating in one direction. If ichthyotic scales also separate in one direction (caudally ?) the name ichthyosis is indeed deserved!

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REFERENCES

ARMUZZI, G. (1962) The therapeutic action of an ointment with a basis of hydrocortisone, lactic acid and calcium pantothenate (Lactacyd Hydrocortisone) in various dermatoses. *Giornale Italiano di Dermatologia*, **103**, 156.

BAKER, H. & BLAIR, C.P. (1968) Cell replacement in the human stratum corneum in old age. *British Journal of Dermatology*, **80**, 367.

BLAIR, C.P. (1968) Morphology and thickness of the human stratum corneum. *British Journal of Dermatology*, **80**, 430.

BRODY, I. (1959) An ultrastructural study of the role of the keratohyalin granules in the keratinization process. *Journal of Ultrastructural Research*, **3**, 84.

CHRISTOPHERS, E. & KLIGMAN, A.M. (1964) Visualization of the cell layers of the stratum corneum. *Journal of Investigative Dermatology*, **42**, 407.

FABRE-TESTE, R. (1958) Acid therapy in lichen tropicus. *Medicine tropical (Madrid)*, **18**, 528.

FEINSTEIN, A., ACKERMAN, A.B. & ZIPRKOWSKI, L. (1970) Histology of autosomal dominant ichthyosis vulgaris and X-linked ichthyosis. *Archives of Dermatology*, **101**, 524.

GRICE, K., SATTAR, H. & BAKER, H. (1973) Urea and retinoic acid in ichthyosis and their effect on transepidermal water loss and water holding capacity of the stratum corneum. *Acta dermato-venereologica*, **53**, 114.

LAGEHOLN, B. & LODN, A. (1966) New acid preparations for skin care. *Lakartidningen*, **63**, 1472.

LUCK, C.P. & WRIGHT, P.G. (1964) Aspects of anatomy and physiology of the skin of the hippopotamus (H. amphibii). *Quarterly Journal of Experimental Physiology*, **49**, 1.

MACKENZIE, J.C. (1969) Ordered structure of the stratum corneum in mammalian skin. *Nature (London)*, **222**, 881.

MARCHIONINI, A. (1934) Sauresalbenbehandling seborrihoischer Hauterktankungen des Gesch Gesichtes. *Deutsche Medizinische Wochenschrift*, **60**, 934.

STERN, E.C. (1946) Topical application of lactic acid in treatment and prevention of certain disorders of skin. *Urologic and Cutaneous Reviews*, **50**, 106.

SWANBECK, G. (1968) A new treatment of ichthyosis and other hyperkeratotic conditions. *Acta dermato-venereologica*, **28**, 123.

WELLS, R.S. & KERR, C.B. (1966) The histology of ichthyosis. *Journal of Investigative Dermatology*, **46**, 530.

British Journal of Dermatology (1976) **94,** 155.

Skin lesions in human yersiniosis

A HISTOPATHOLOGICAL AND IMMUNOHISTOLOGICAL STUDY

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SUMMARY

Various types of skin manifestations of human yersiniosis were examined with routine histological and immunohistological methods. The biopsy material consisted of sixteen cases of erythema nodosum, eight cases of erythema multiforme, and one case of erythema figuratum. The principal histopathological changes in erythema nodosum were septal or diffuse, mild panniculitis and in seven cases also necrotizing vasculitis in small, medium-sized or large arteries. Perivascular lymphocytic infiltration without vasculitis was the most prominent feature in erythema figuratum.

By using a polyvalent conjugate, immunoglobulins in vessel walls in the dermis were found in two cases of EM. The rapid course of the skin eruptions and the frequency of necrotizing vasculitis in arteries fit the changes seen in an experimental Arthus reaction, in which necrotizing vasculitis is followed by lymphocytic inflammation consistent with a delayed type of reaction.

Infections caused by *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* are usually manifested by gastrointestinal symptoms often mimicking acute gastroenteritis, but also acute appendicitis and mesenteric lymphadenitis (Ahvonen, 1972). Quite often, yersiniosis also produces skin rashes, such as erythema nodosum (EN), as is well known in the Scandinavian countries and in France (Ahvonen, 1972; Kerzoncuf, 1967; Mollaret, 1971; Nilehn *et al.*, 1968; Winblad, 1969), and sometimes erythema multiforme (EM) (Hannuksela & Ahvonen, 1975), exanthemata (Delorme *et al.*, 1974; Hannuksela & Ahvonen, 1975), and erythema figuratum (EF) (Hannuksela & Ahvonen, 1975).

This study, using routine histological and immunohistological methods, aims at shedding more light on the pathophysiology of the skin lesions induced by yersinia infections.

PATIENTS AND METHODS

Twenty-five biopsies were taken from various kinds of skin lesions of twenty patients with acute human yersiniosis. In five cases biopsies were taken of both EN and EM lesions from the same patients.

TABLE I. Some clinical and laboratory data on twenty patients with human yersiniosis

Patient no.	Age	Sex	Types of skin lesion*	Causative strain†	Isolation of bacteria from faeces	Highest antibody titre	Preceding symptoms and signs‡	Biopsy			
								Type of lesion	Site	Interval (days) between biopsy and: prec. symptoms	skin rash
1	35	F	EN, EM	Y. ent. 3	–	640	Resp.	EN	Arm	21	8
2	24	F	EN	Y. ent. 3	–	640	—	EN	Leg	—	8
3	51	F	EN, EM	Y. ent. 3	–	320	—	EN	Leg	—	6
								EM	Arm	—	6
4	38	F	EN	Y. ent. 3	+	20480	Abd.	EN	Leg	4	2
5	61	F	EN	Y. ent. 3	–	2560	Abd.	EN	Leg	17	9
6	46	F	EN, EM	Y. ent. 3	–	1280	Abd.	EM	Arm	13	5
7	36	F	EN, EM	Y. ent. 3	+	5120	Headache	EN	Leg	7	5
								EM	Arm	7	5
8	33	F	EN	Y. ent. 3	–	2560	Abd.	EN	Leg	29	16
9	38	F	EN, EM	Y. ent. 3	–	160	Abd.	EN	Leg	12	3
								EM	Leg	12	3
10	33	F	EN	Y. ent. 9	–	2560	Abd.	EN	Thigh	11	5
11	62	F	EN, EM	Y. ent. 9	+	160	Abd.	EM	Thigh	25	5
12	22	F	EN	Y. ent. 9	+	80	—	EN	Leg	—	6
13	44	F	EN	Y. ent. 9	–	640	Abd.	EN	Leg	8	5
14	33	F	EN	Y. ent. 9	–	1280	—	EN	Thigh	—	18
15	21	F	EN	Y. ent. 9	–	320	Abd.	EN	Leg	18	7
16	57	F	EN, EM	Y. ent. 9	+	320	Resp.	EM	Arm	31	10
17	60	F	EN, EM	Y. ent. 9	+	2560	Headache	EN	Arm	8	5
18	29	F	EN, EM	Y. ent. 9	–	1280	Abd.	EN	Leg	11	7
								EM	Thigh	11	7
19	45	F	EN, EF	Y. ent. 9	–	640	Arthritis	EF	Arm	35	25
20	33	M	EN, EM	Y. pstb.	–	160	Abd.	EN	Leg	6	3
								EM	Arm	6	3

* EN = erythema nodosum; EM = erythema multiforme; EF = erythema figuratum.

† Y. ent. 3 = Yersinia enterocolitica type 3; Y. ent. 9 = Yersinia enterocolitica type 9; Y. pstb. = Yersinia pseudotuberculosis type I.

‡ Resp. = common cold type acute respiratory disease; Abd. = abdominal colic or pain and diarrhoea; – = no preceding symptoms.

The biopsies were taken under local anaesthesia. One half of each specimen was fixed in formalin for histological study and the other half was snap-frozen for immunohistological studies. The histological procedure consisted of embedding in paraffin, cutting, and staining with haematoxylin–eosin, methyl green–pyronin and resorcin–fuchsin. For the immunohistological examination, the biopsies were cut, stained and examined as described earlier (Salo, 1970).

The diagnosis of yersiniosis was based on a raised antibody titre (160 or more) or on demonstration of the bacteria from faeces. The clinical and some laboratory data are given in Table 1. EN only was diagnosed in nine cases, both EN and EM in ten cases, and both EN and EF in one case.

RESULTS

Sixteen lesions of EN, eight of EM (Figs 1 and 2) and one of EF were examined. In the histological examination the inflammation was most prominent in the subcutis in seventeen lesions and in the dermis in eight lesions (Table 2). In ten cases the subcutaneous inflammation was characterized by

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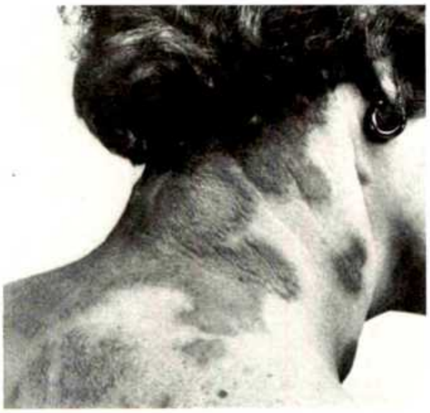

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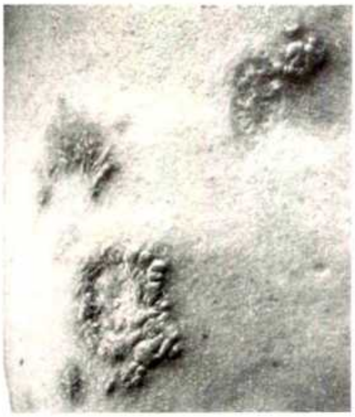

FIGURE 1. Case no. 7. Cockade-like erythema multiforme on the neck and shoulders.

FIGURE 2. Seven days later the same patient as in Fig. 1. developed a vesicular form of erythema multiforme on her upper arms.

TABLE 2. Histopathological reaction patterns of skin eruptions in human yersiniosis

	Histological findings			
	Panniculitis		Lymphocytic inflammation in the dermis	
Clinical type of eruption	Without vasculitis	With vasculitis	Without oedema	With oedema
Erythema nodosum	7 (1, 7, 8, 12, 13, 18, 20)*	8 (2, 3, 4, 5, 9, 10, 14, 15)*	1 (17)*	—
Erythema multiforme	2 (11, 20)*	—	2 (9, 16)*	4 (3, 6, 7, 18)*
Erythema figuratum	—	—	1 (19)*	—

* Case numbers.

broad connective tissue septa with few adjacent inflammatory round cells. In seven cases the subcutaneous tissue showed only mild diffuse inflammation characterized by the proliferation of small capillaries between the fat cells. In both the septal and diffuse types of panniculitis small tight masses of lymphocytes and in some cases neutrophils could occasionally be found. Necrotizing vasculitis of large and medium-sized arteries was found in five lesions (Fig. 3), and of small vessels in two lesions (Fig. 4). A vein was found to be inflamed in only one case.

The dermal inflammation appeared in two slightly different forms (Table 2). In four cases there was a tight perivascular infiltration of small lymphocytes in the upper or middle dermis, and in four additional cases this was combined with dermal oedema in the papillary layer. In two of these the

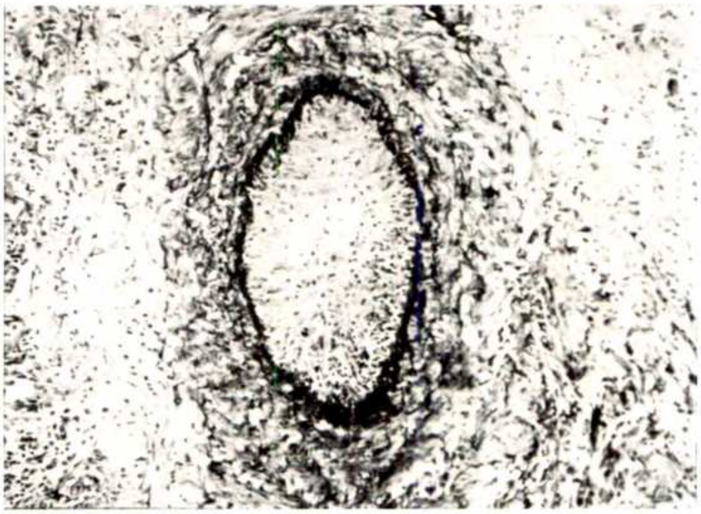

FIGURE 3. Erythema nodosum on the leg with necrotizing arteritis (case no. 5) (Resorcin–Fuchsin ×400).

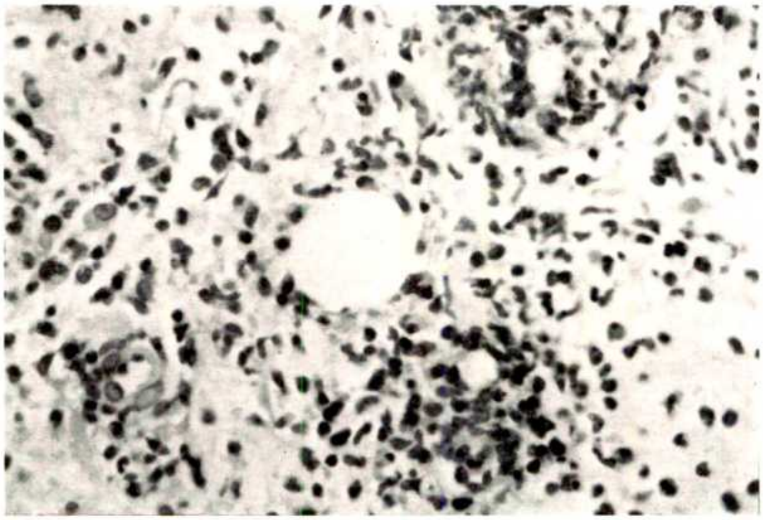

FIGURE 4. Erythema nodosum on the thigh (case no. 10). Necrotizing vasculitis of small vessels on the dermo–subcutaneous border (H & E, ×400).

lymphocytic infiltrate was mixed with polymorphonuclear leukocytes. In no case were eosinophils or plasma cells (Methyl green–pyroninophils) found. Vasculitis was not found in the dermal inflammation.

Perivascular lymphocytic infiltrations with or without subepidermal or perivascular oedema were typical features both in the vesicular form (five lesions) and in the cockade-like form (three lesions) of EM. In the only case of yersinia EF, perivascular lymphocytic infiltration but no oedema was found in the dermis (Fig. 5).

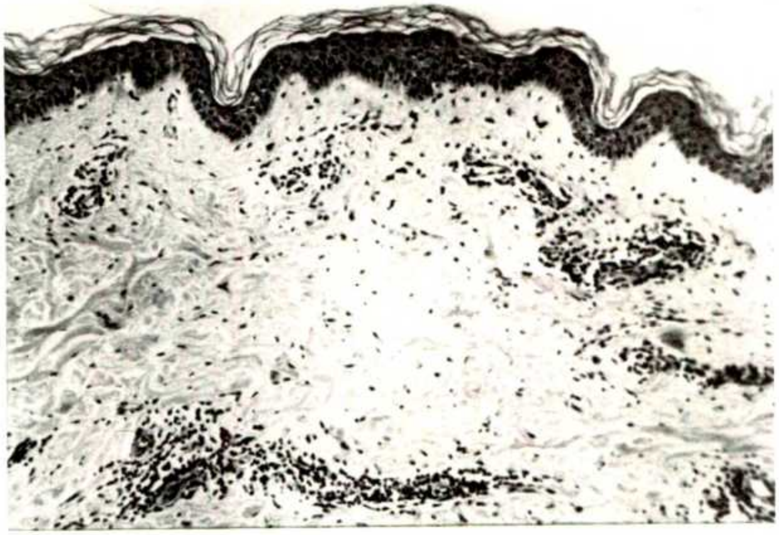

FIGURE 5. Erythema figuratum (case no. 19). Sleeve-like infiltrate of lymphocytes in the upper part of the dermis (H & E, × 100).

The immunohistological examination revealed precipitation of immunoglobulins in the walls of dermal vessels in two cases (Nos 6 and 7), which were clinically classified as EM. Histologically these were the cases with a mixed inflammatory infiltrate and oedema. The different histological or immunohistological findings did not correlate with the antibody titre or with the age of the skin lesion.

DISCUSSION

The combination of EN and EM with yersiniosis is more usual than that with other causes underlying EN (Hannuksela & Ahvonen, 1975). According to the classical concept, the consistent histopathological feature in EN is septal panniculitis with accumulations of lymphocytes in the septa of the panniculus adiposus. Sometimes non-necrotizing vasculitis can also be seen in large veins (Löfgren & Wahlgren, 1949). The present results fit this concept only in part. In ten cases of EN septal panniculitis was present, but in seven cases the inflammation in the adipose tissue was diffuse and mild, and all were characterized by small discrete accumulations of lymphocytes. In addition, necrotizing arteritis in the subcutis was seen in seven of sixteen cases of EN.

EM, figurate erythemas and exanthemata belong to the large group typified by a dermal perivascular lymphocytic infiltration without vasculitis. The histological picture of the lesions classified clinically as EM in the present study is very close to EM as described by others (Pinkus & Mehregan, 1969; Orfanos, Schaumburg-Lever & Lever, 1974; Reed & Clark, 1971). The fact that in two of our eight cases of EM the subcutis was inflamed (Table 2) demonstrates the overlapping of EM and EN.

EN has been considered a manifestation of a delayed hypersensitivity reaction influenced by mixed infections and other provocative factors (Löfgren, 1946; Westergren, 1946). Damage caused by antigen–antibody reactions is also said to be of pathogenetic significance (Borrie, 1970; Fine & Meltzer, 1968). Parish & Rhodes (1967) have demonstrated immunoglobulins and bacterial antigens in lesions of nodular vasculitis, and Scott & Rowell (1965) have demonstrated immunoglobulins in the lesions of EN. Whether the presence of immunoglobulins in two of our eight cases of EM is a sign of an Arthus reaction or not cannot be resolved. The simultaneous appearance of EN and EM after various intervals from the first clinical signs and symptoms of yersiniosis suggests that both types of reactions are only different manifestations of the same immunological host response to *Yersinia* bacteria. In the experimental Arthus reaction, necrotizing vasculitis is followed by lymphocytic inflammation consistent with a delayed type of reaction (Cream, Bryceson & Ryder, 1971). The skin rash in yersiniosis may be induced by antigen–antibody complexes which disappear very quickly, while the reaction then continues as a lymphocytic infiltration as in the experimental Arthus reaction.

REFERENCES

AHVONEN, P. (1972) Human yersiniosis in Finland. II. Clinical features. *Annals of Clinical Research*, **4**, 39.

BORRIE, P. (1970) Cutaneous vasculitis. *Proceedings of the Royal Society of Medicine*, **63**, 815.

CREAM, J.J., BRYCESON, A.D.M., & RYDER, G. (1971) Disappearance of immunoglobulin and complement from the Arthus reaction and its relevance to studies of vasculitis in man. *British Journal of Dermatology*, **84**, 106.

DELORME, J., LAVERDIÈRE, M., MARTINEAU, B. & LAFLEUR, L. (1974) Yersiniosis in children. *Canadian Medical Association Journal*, **110**, 281.

FINE, R.M. & MELTZER, H.D. (1968) Erythema nodosum: a form of allergic cutaneous vasculitis. *Southern Medical Journal*, **61**, 680.

HANNUKSELA, M. & AHVONEN, P. (1975) Skin manifestations in human yersiniosis. *Annals of Clinical Research*, in press.

KERZONCUF, A. (1967) L'érythème noueux à bacille de Malassez et Vignal. *Thesis*. Copedith, Paris.

LÖFGREN, S. (1946) Erythema nodosum. Studies on etiology and pathogenesis of 185 adult cases. *Acta Medica Scandinavica*, supplement **174**.

LÖFGREN, S. & WAHLGREN, F. (1949) On the histopathology of erythema nodosum. *Acta dermato-venereologica*, **29**, 1.

MOLLARET, H.H. (1971) L'infection humaine à Yersinia enterocolitica en 1970, à la lumière de 642 cas récents. Aspects cliniques et perspectives épidémiologiques. *Pathologie-Biologie (Paris)*, **19**, 189.

NILÉHN, B., SJÖSTRÖM, B., DAMGAARD, K. & KINDMARK, C. (1968) Yersinia enterocolitica in patients with symptoms of infectious diseases. *Acta Pathologica et Microbiologica Scandinavica*, **74**, 101.

ORFANOS, C.E., SCHAUMBURG-LEVER, G. & LEVER, W.F. (1974) Dermal and epidermal types of erythema multiforme. A histologic study of 24 cases. *Archives of Dermatology*, **109**, 682.

PARISH, W.E. & RHODES, E.L. (1967) Bacterial antigens and aggregated gamma globulin in the lesions of nodular vasculitis. *British Journal of Dermatology*, **79**, 131.

PINKUS, H. & MEHREGAN, A.H. (1969) *A Guide to Dermato-histopathology*, pp. 130–137. Appleton-Century-Crofts, New York.

REED, J.R. & CLARK, W.H. (1971) Basic pathologic reactions of the skin. In: *Dermatology in general medicine* (Ed. by T.B.Fitzpatrick, K.A.Arndt, W.H.Clark, A.Z.Eisen, E.J.van Scott and J.H.Vaughan), pp. 192–211. McGraw-Hill, New York.

SALO, O.P. (1970) SLE-like deposition of immunoglobulins in the skin in rosacea. *Annals of Clinical Research*, **2**, 28.

SCOTT, D.G. & ROWELL, N.R. (1965) Preliminary investigations of arteric lesions using fluorescent antibody techniques. *British Journal of Dermatology*, **77**, 211.

WESTERGREN, A. (1946) On a complex etiology of erythema nodosum with reference to the results of an odontologic study. *Acta dermato-venereologica*, **26**, 384.

WINBLAD, S. (1969) Erythema nodosum associated with infection with Yersinia enterocolitica. *Scandinavian Journal of Infectious Diseases*, **1**, 11.

British Journal of Dermatology (1976) 94, 161.

Skin surface lipid composition in rosacea

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Skin surface lipid composition was measured in thirty-one patients with rosacea and their age-matched controls. Patients with rosacea had normal lipid composition, and there was no correlation between the lipid composition and the severity of the disease. Tetracycline therapy produced no measurable change in lipid composition.

There is some clinical similarity between the papules of acne vulgaris and rosacea. Tetracycline has a beneficial effect in both conditions, and in acne vulgaris this effect has been attributed to a reduction in free fatty acids (FFA) (Frienkel *et al.*, 1965). FFA have been shown to be irritant when injected into the skin (Strauss & Pochi, 1965), and when applied to skin under occlusion (Kellum, 1968). It is generally accepted that the pilosebaceous unit is affected in rosacea (Milne, 1972), but the sebum excretion rate is normal (Burton *et al.*, 1975), and the cause of rosacea remains unknown. We have, therefore, studied lipid composition before and after tetracycline therapy in this condition.

METHOD

The skin surface lipid composition was measured by thin layer chromatography (TLC) in thirty-one patients with rosacea, aged 26–74 years, and their age-matched controls. Patients with a previous history of acne vulgaris were not excluded from either group as this would have biased our results if rosacea has either a positive or a negative association with acne vulgaris. The degree of rosacea was judged on clinical grounds and classed as mild, moderate, or severe—depending on the appearance of the forehead skin. There were eleven patients with rosacea who were studied before and after 2 months' treatment with oxytetracycline (250 mg twice daily). The lipid was collected from the forehead skin, which had been prepared by applying absorbent paper repeatedly until only the follicular pattern appeared on the paper (Cunliffe & Shuster, 1969). A glass cylinder of 1·5 cm diameter was applied firmly to the prepared skin. Ether was pipetted into the cylinder and left for 2 mins. Samples in ether were taken from both sides of the forehead in each patient. This technique was used in preference to the paper method (Cotterill *et al.*, 1971) as it has been shown that the FFA may be strongly bound to some component of paper and not readily removed by fat solvents (Strauss *et al.*, 1964; Kanaar, 1971) Samples were stored in ether at 0°C and analysed by TLC within 24 h.

The TLC method used was as follows: A 20 × 20 cm plate, spread with a mixture of Keiselgel G and 5% ammonium sulphate and allowed to dry (Walker, 1971), was washed in ether for 1 h to remove lipid contaminants and then activated at 100°C for 1 hr. The lipid sample in ether was then evaporated under N_2 at 38°C, and the residue was taken up in 0·5 ml of chloroform. 20 μl aliquots were spotted on the plate and developed in three successive solvent systems. First pet. ether 60/40: benzene in a ratio of 1:1 was run to 18 cm and allowed to dry; this was repeated. Then ether: pet. ether 60/40: glacial

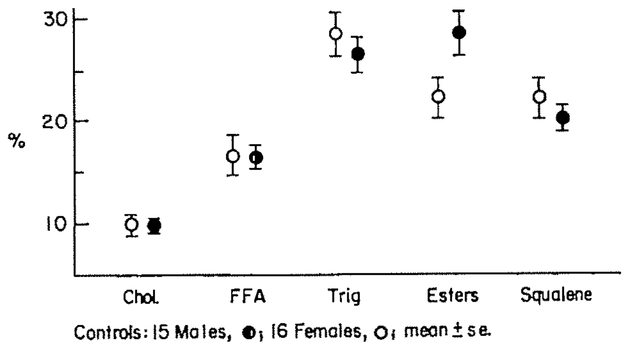


FIGURE 1. Skin surface lipid composition (expressed as percentages) in male and female control subjects.

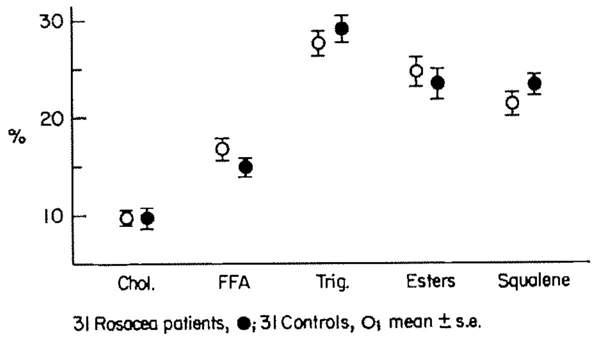


FIGURE 2. Skin surface lipid composition in patients with rosacea and in control subjects matched for age and sex.

acetic acid in a ratio of 13:7:0·1 was run to 8 cm. After drying the spots were charred at 200°C for 1 h and read on a photodensitometer (Joyce-Loebl Chromoscan 200).

RESULTS

We examined skin surface lipids in 15 male and 16 female controls, and found that there was no difference in surface lipids between the sexes; the lower value for esters in females is not statistically significant (Fig. 1).

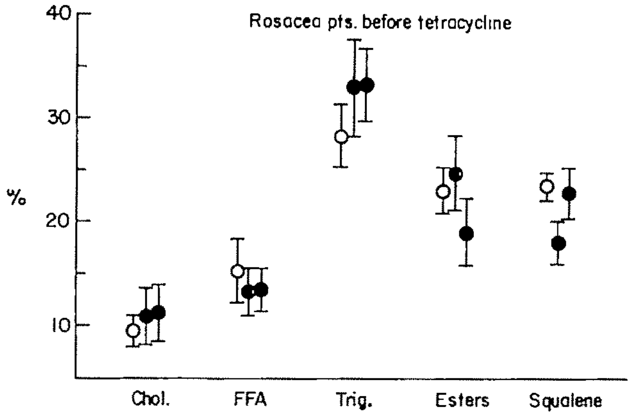
Fig. 2 again shows the components or surface lipids expressed as a percentage of total lipid. It can be seen that there is no difference in the results of patients with rosacea and their age-matched controls.

In Fig. 3 we compared the surface lipids in patients with varying severity of disease. There was no statistical difference in results obtained in any of the groups.

There were eleven patients with rosacea who were studied before and after tetracycline (Fig. 4). The results show that the surface lipids in these patients were not significantly altered by tetracycline therapy.

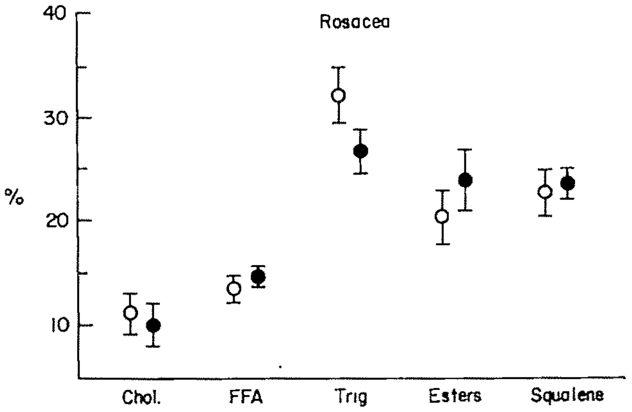
DISCUSSION

Rosacea bears a clinical resemblance to acne vulgaris and this, together with the occurrence of sebaceous gland hyperplasia in rhinophyma and the histological similarity between rosacea and acne vulgaris (Lever, 1967; Allen, 1966; Fitzpatrick *et al.*, 1971), has led to the widely accepted view that rosacea is a disorder of the pilosebaceous units (Fitzpatrick *et al.*, 1971). This view has, however, been disputed by Marks & Harcourt-Webster (1969) who studied the histology of rosacea in 108 patients. In papular



7 mild ○; 5 mod. ●; 8 severe ●.

FIGURE 3. Skin surface lipid composition in patients with rosacea of varying degrees of severity.



11 pts before tetracyline, ○; 11 pts. after tetracyline, ●; mean ± s.e.

FIGURE 4. Skin surface lipid composition in patients with rosacea before and after tetracycline therapy.

rosacea the inflammatory infiltrate was partially distributed around the hair follicles in 51% of biopsy specimens, and in serial sections only 25% showed areas of folliculitis. They therefore concluded that there is no basic follicular abnormality in rosacea, and any apparent involvement of the follicles is coincidental.

Our present study has shown that the skin surface lipid composition is normal in rosacea, and we have found no decrease in FFA following clinical improvement with tetracycline therapy. We therefore feel that gross changes in FFA cannot be implicated in the pathogenesis of rosacea, although we have not excluded the possibility of small changes in the concentration of potent inflammatory chemicals such as prostaglandins. The Meibomian glands of the eyelids are modified sebaceous glands, and Cory *et al.* (1973) have failed to detect any difference in Meibomian gland lipid composition between control subjects and patients with the red eyes associated with rosacea. The two studies, together with our recent report that seborrhoea is not a feature of rosacea (Burton *et al.* 1975), are compatible with the idea that rosacea is not primarily a disorder of the pilosebaceous apparatus.

It has been shown that the sampling technique (paper, sponge or cup) affects the results of skin surface lipid analysis (Cunliffe, Cotterill & Williamson, 1971). We believe that the time interval between cleansing and collection of the lipid sample is equally important. The cholesterol is derived almost entirely from the epidermis (Ramasastry *et al.*, 1970) and the squalene is derived from both epidermal lipid and sebum (Nicholaides & Rothman, 1955). As the time interval between cleansing and collection is prolonged, so the proportion of sebum to epidermal lipid increases. We feel that this may explain the relatively high proportion of cholesterol and squalene we found, compared with the results of Downing, Strauss & Pochi (1969). This is supported by Wilkinson (1969) who studied skin surface lipid of forearm skin at varying intervals after cleansing. His findings at 1½ h resembled ours, whereas at 3 h they were similar to the results of Downing *et al.* (1969). We are currently undertaking further work to elaborate this point.

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REFERENCES

ALLEN, A.C. (1966) *The Skin*, 2nd. edn, p. 735. Grune & Stratton, New York.

BURTON, J.L., PYE, R.J., MEYRICK, G. & SHUSTER, S. (1975) The sebum excretion rate in rosacea. *British Journal of Dermatology*, **92**, 541.

CORY, C.C., HINKS, W., BURTON, J.L. & SHUSTER, S. (1973) Meibomian gland secretion in the red eyes of rosacea. *British Journal Dermatology*, **89**, 25.

COTTERILL, J.A., CUNLIFFE, W.J., WILLIAMSON, B. & FORSTER, R.A. (1971) A semiquantitative method for the biochemical analysis of sebum. *British Journal of Dermatology*, **85**, 35.

COTTERILL, J.A., CUNLIFFE, W.J., WILLIAMSON, B. & BULUSU, L. (1972) Age and sex variation in skin surface, lipid composition and sebum excretion rate. *British Journal of Dermatology*, **87**, 333.

CUNLIFFE, W.J. & SHUSTER, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697.

CUNLIFFE, W.J., COTTERILL, J.A. & WILLIAMSON, B. (1971) Variations in skin surface lipid composition with different sampling techniques. *British Journal of Dermatology*, **85**, 40.

DOWNING, D.T., STRAUSS, J.S. & POCHI, P.E. (1969) Variability in the chemical composition of human skin surface lipids. *Journal of Investigative Dermatology*, **53**, 322.

FITZPATRICK, T.B. *et al.* (1971) *Dermatology in general medicine*, p. 370. McGraw-Hill, New York.

FREINKEL, R.K., STRAUSS, J.S., YIP, S.Y. & POCHI, P.E. (1965) Effect of tetracycline on the composition of sebum in acne vulgaris. *New England Journal of Medicine*, **273**, 851.

KANAAR, P. (1971) Variation in skin surface lipid composition. *British Journal of Dermatology*, **85**, 600.

KELLUM, R.E. (1968) Acne vulgaris. Study in pathogenesis: relative irritancy of free fatty acids from C2 to C16. *Archives of Dermatology*, **97**, 722.

LEVER, W.F. (1967) *Histopathology of skin*, 4th edn, p. 189. Lippincott. Philadelphia.

MARKS, R. & HARCOURT-WEBSTER, J.N. (1969) Histopathology of rosacea. *Archives of Dermatology*, **100**, 683.

MILNE, J.A. (1972) *An introduction to the diagnostic histopathology of the skin*, p. 113. Edward Arnold, London.

NICHOLAIDES, N. & ROTHMAN, S. (1955) The site of steroid and squalene synthesis in human skin. *Journal of Investigative Dermatology*, **24**, 125.

RAMASASTRY, P., DOWNING, D.T., POCHI, P.E. & STRAUSS, J.S. (1970) Chemical composition of human skin surface lipids from birth to puberty. *Journal of Investigative Dermatology*, **54**, 139.

STRAUSS, J.S., POCHI, P.E., MASUCCI, F.J. & MATTHENY, E.J. (1964) Titratable acidity of sebum as determined by a micropotentiometric technique. *Journal of Investigative Dermatology*, **42**, 349.

STRAUSS, J.S. & POCHI, P.E. (1965) Histological observations following intracutaneous injection of sebum and comedones. *Archives of Dermatology*, **92**, 443.

WALKER, B.L. (1971) A novel charring technique for detection of lipids on thin layer chromatograms. *Journal of Chromatography*, **56**, 320.

WILKINSON, D.I. (1969) Variability in composition of surface lipids. *Journal of Investigative Dermatology*, **52**, 339.

British Journal of Dermatology (1976) **94**, 165.

Sebaceous lipogenesis in human skin

VARIABILITY WITH AGE AND WITH SEVERITY OF ACNE

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SUMMARY

Lipogenesis from [^{14}C] glucose was measured in skin biopsies from the shoulder-blade region of forty-two male subjects. In the acne age range, dermal lipogenesis showed an upward trend with increasing severity of acne; a similar trend was found in forehead sebum excretion rate, but no correlation was obvious between dermal lipogenesis (back) and sebum excretion rate (forehead) in the same subject. In older subjects, dermal lipogenesis was significantly increased in those with a past history of severe acne.

At high rates of dermal lipogenesis there was a small but significant increase in wax ester labelling relative to triglyceride and squalene. No increase was found in squalene labelling relative to other lipids, either with increasing lipogenic rate or in severe acne.

Dermal and epidermal lipogenesis rates showed no correlation with age. In dermis, however, there was a decrease in wax ester labelling relative to triglyceride, and a marked decrease in squalene labelling relative to both triglyceride and wax esters, with increasing age.

The relationship between sebum excretion rate (SER) and clinical severity of acne is now well established (Pochi & Strauss, 1964; Cunliffe & Shuster, 1969a; Burton & Shuster, 1971; Cotterill, Cunliffe & Williamson, 1971). The question of whether acne is also associated with a change in sebum composition has been explored by many workers, and while some studies have shown no significant differences in surface lipid (Boughton *et al.*, 1959; Pochi & Strauss, 1964; Runkel, Wurster & Cooper, 1969; Salamon *et al.*, 1970; Fulton, Anderson & Randall, 1971; Lewis & Hayward, 1971), other workers have reported an increased proportion of wax esters (Powell & Beveridge, 1970; Cotterill *et al.*, 1972) and squalene (Cotterill *et al.*, 1972; Gloor *et al.*, 1972), and a change in the fatty acid composition of triglyceride (Kellum & Strangfeld, 1972; Krakow *et al.*, 1973).

The study of sebaceous gland activity by measuring surface lipid composition is subject to difficul-

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ties of sampling (Downing, 1974) and bacterial decomposition. For these reasons we have made more direct studies of lipogenesis by the sebaceous glands.

MATERIALS AND METHODS

The subjects for this study were forty-two male volunteers aged between 16 and 68 years. Those in the age range 16–26 were grouped according to clinical severity of acne into grades 0–4 (Cunliffe & Shuster, 1969a). No subjects were included whose clinical grading had changed as a result of tetracycline therapy. Subjects in the age range 27–68 were classified into those with a past history of severe acne and those without. The 'control' group in this age range included eight subjects with minor skin disorders (warts, leg ulcers, alopecia areata, etc.) and nine subjects with psoriasis; no significant differences in dermal lipogenesis were found between the psoriatics and the other control subjects. Three patients with psoriasis were also included in the grade 0/1 groups of the 16–26 age range.

Two 4 mm punch biopsies were taken using a high-speed drill, without local anaesthetic, from lesion-free areas in the scapular region of the back. Subcutaneous adipose tissue was carefully trimmed away and the intact biopsies were incubated for 3 h at 37°C in Krebs Ringer Bicarbonate containing benzylpenicillin (100 μg/ml), streptomycin sulphate (100 μg/ml) and 2 mM [U-^{14}C] glucose (5 mCi/mmol). By using glucose, in substrate quantities, as the labelled precursor it was hoped to avoid artifacts which might arise from the use of a tracer (such as acetate) to label a precursor pool of potentially variable size. There is evidence that glucose incorporation under these conditions parallels the absolute rate of lipogenesis (Wheatley *et al.*, 1971).

After incubation the biopsies were soaked for 2½–3 h in 0·5% acetic acid to loosen the epidermis, which could then be peeled away leaving the sebaceous glands *in situ* in the dermis. Total lipids were extracted from the tissue and separated into classes by one-dimensional thin layer chromatography on silicic acid. Radioactivity (dpm per extract) was measured by scintillation counting. Further details of the method have been reported elsewhere (Cooper, Thody & Shuster, 1974; Cooper, McGrath & Shuster, 1976).

Sebum excretion rate (forehead) was measured as described by Cunliffe & Shuster (1969b).

Nonparametric statistical methods were taken from Siegel (1956). For correlations against acne grade the Spearman r_s was used as being numerically similar to the Pearson r. For correlations involving ratios, where it was felt that parametric assumptions might be inappropriate, the Kendall correlation was used instead because it can be generalized to a partial correlation coefficient. Elsewhere, parametric regressions were used.

RESULTS

Dermal and epidermal lipogenesis in acne

In subjects aged between 16 and 26 years, ^{14}C incorporation into total dermal lipids showed an upward trend with increasing severity of acne (Fig. 1; Spearman $r_s = 0{\cdot}258$), but there was considerable overlap of individual values between groups. In the same subjects the correlation of forehead sebum excretion rate with severity of acne was somewhat greater ($r_s = 0{\cdot}458$). No correlation was obvious between sebum excretion rate (forehead) and dermal lipogenesis (back) in the same subject.

In a small group of older subjects (aged from 29 to 51 years) with a past history of severe acne, and with continuing acneiform lesions, the rate of dermal lipogenesis was significantly higher than in control subjects in the same age range (Fig. 2; $P < 0{\cdot}01$).

Epidermal lipogenesis showed no correlation with severity of acne, and no difference was found between 'post-acne' subjects and controls.

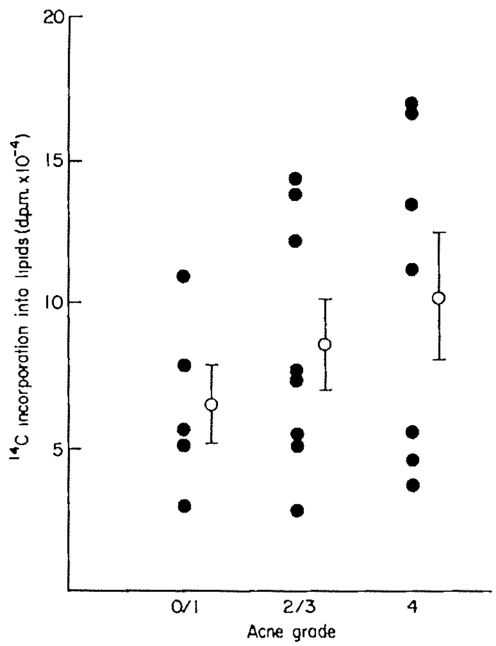

FIGURE 1. Correlation of dermal lipogenesis rate (dpm per biopsy) with severity of acne in subjects aged 16–26 years. Closed circles are individual values and open circles represent mean ± s.e. mean.

Lipogenic pattern in relation to overall rate

Fig. 3 shows the mean distribution of ^{14}C between lipid classes in dermis, in the 16–26 years age group. Most of the isotope was recovered in triglyceride, squalene, wax esters/sterol esters and polar lipids, with smaller quantities appearing in free fatty acids, sterols and the partial glycerides.

At higher overall rates of lipogenesis, the labelling of neutral lipids tended to increase relative to polar lipids. Thus there was a positive correlation between the percentage of isotope appearing in neutral lipid and the total lipogenic rate ($r = 0{\cdot}448$, $P < 0{\cdot}05$). This effect was quantitatively small, with the regression line for neutral lipids showing an increase from 90 to 93% of total over the whole range of activity.

In order to see whether the proportions of the three main secretory lipids (triglyceride, squalene and wax esters) were altered relative to each other, the ratios wax ester/triglyceride, wax ester/squalene and squalene/triglyceride were calculated. Table 1 shows the nonparametric correlation coefficients for these values against lipogenic rate. Wax ester labelling increased relative to both triglyceride and squalene as the rate of total lipid labelling increased; the ratio of squalene/triglyceride, however, showed no change. Calculation of partial correlation coefficients to control out the effect of age did not substantially alter these results (Table 1). In absolute terms, the increase in wax ester labelling at higher lipogenic rates was small, and the regression line for monoesters (as percentage of neutral lipids) varied between 9·8 and 12·6% over the whole range of lipogenic rates.

Effects of age on dermal and epidermal lipogenesis

Dermal and epidermal lipogenesis rates showed no correlation with age. In dermis, however, the pattern of lipid labelling altered with increasing age, the most striking change being a reduction in the percentage labelling of squalene (Fig. 4a). Wax ester labelling was also slightly reduced (Fig. 4a);

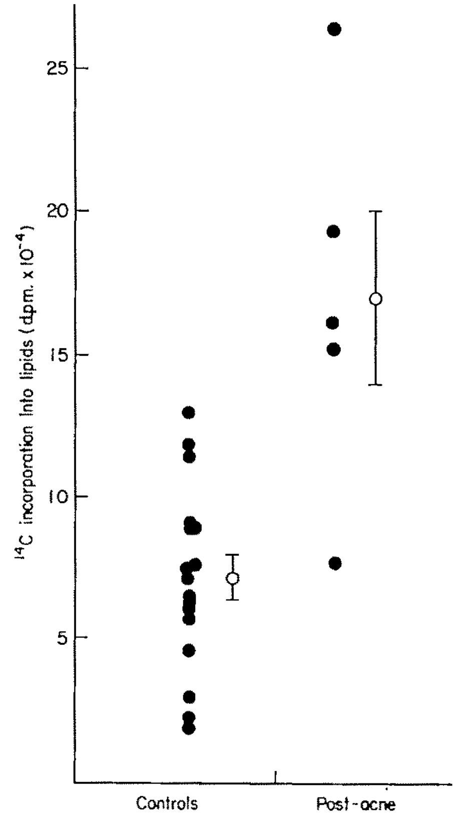

FIGURE 2. Dermal lipogenesis rate (dpm per biopsy) in control subjects aged 27–68 years and in subjects aged 29–51 years with a past history of severe acne. Closed circles are individual values and open circles represent mean ± s.e. mean.

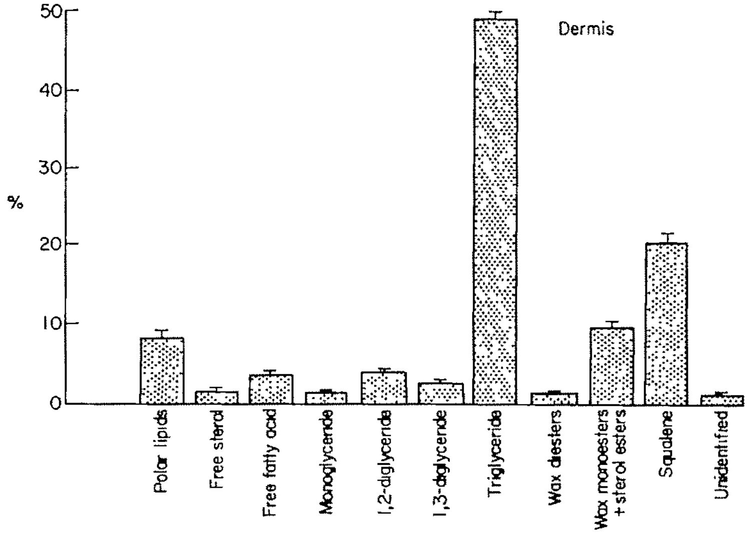

FIGURE 3. Distribution of ^{14}C between dermal lipid classes in skin from subjects aged 16–26 years. Bars represent s.e. mean (n = 20).

TABLE 1. Variation in pattern of lipogenesis with overall rate

Ratio	Kendall correlation coefficient	P	Partial correlation coefficient (allowing for age)
Wax esters + sterol esters/triglycerides	+0·186	<0·05	+0·203
Wax esters + sterol esters/squalene	+0·156	NS (P<0·1)	+0·141
Squalene/triglycerides	+0·001	NS	+0·043

Ratios of labelling in different lipid classes were correlated with total incorporation of isotope into dermal lipids (dpm/biopsy/3 h) using the Kendall rank correlation. Partial correlation coefficients were then calculated to exclude the effect of age (see Table 2). (N.B. The underlying scale of the Kendall coefficient is not equivalent to that of the Pearson r.)

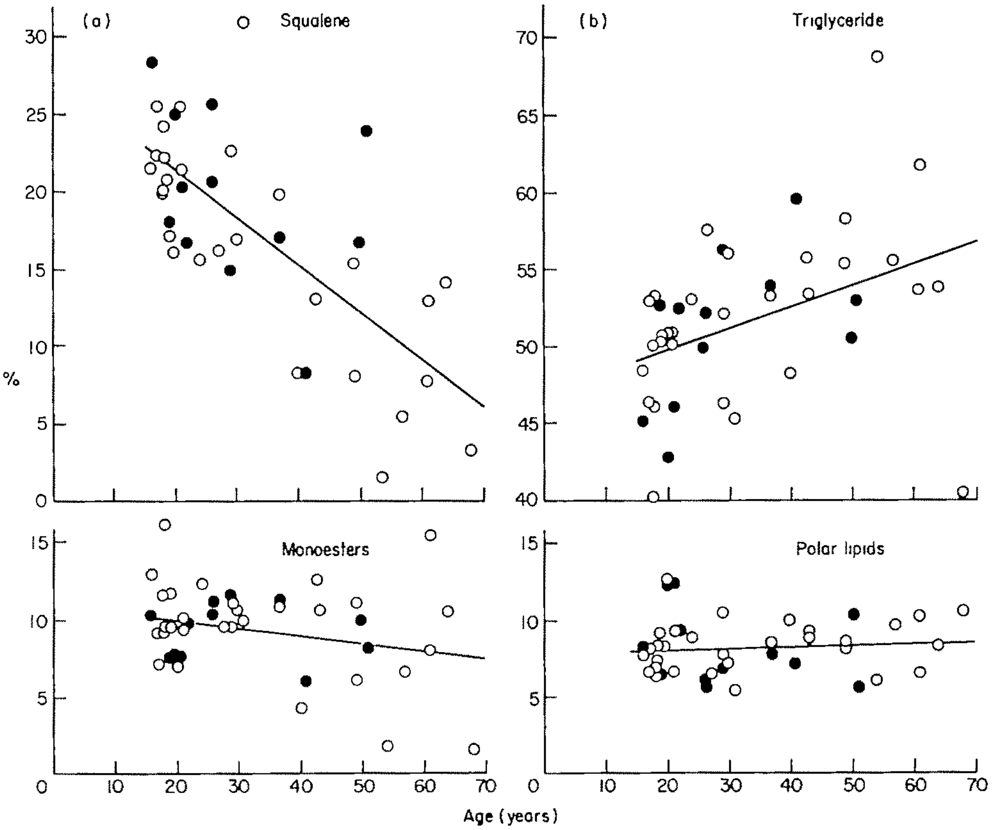

FIGURE 4. (a) Correlation of squalene and wax monoester/sterol ester labelling (each as percentage of total lipids) with age. Closed circles are grade 4 acne or 'post-acne'. Linear regressions are shown ($r = -0{\cdot}707$, squalene; $r = -0{\cdot}297$, wax esters).

(b) Correlation of triglyceride and polar lipid labelling (each as percentage of total lipids) with age. Closed circles are grade 4 acne or 'post-acne'. Linear regressions are shown ($r = +0{\cdot}409$, triglyceride; $r = +0{\cdot}083$, polar lipids).

TABLE 2. Variation in pattern of lipogenesis with age

Ratio	Kendall correlation coefficient	P	Partial correlation coefficient (allowing for lipogenic rate)
Squalene/wax esters + sterol esters	−0·415	<0·0001	−0·410
Squalene/triglycerides	−0·532	<0·0001	−0·533
Wax esters + sterol esters/triglycerides	−0·193	<0·05	−0·210

Ratios of labelling in different lipid classes were correlated with age using the Kendall rank correlation. Partial correlation coefficients were then calculated to exclude the effect of lipogenic rate (see Table 1). (N.B. The underlying scale of the Kendall coefficient is not equivalent to that of the Pearson r.)

triglyceride labelling was increased (Fig. 4b), while the percentage of polar lipids remained fairly constant (Fig. 4b). Table 2 shows that squalene labelling decreased relative to wax esters, and both squalene and wax ester labelling decreased relative to triglyceride. The changes in squalene, relative to both the other lipid classes, were highly significant. Partial correlation coefficients indicate that these changes were independent of the effect of lipogenic rate on isotope distribution.

DISCUSSION

The results presented here indicate that lipogenesis from glucose in the dermis is raised in the two situations so far examined in which the sebum excretion rate is high, namely in acne patients and in subjects with a past history of severe acne. Autoradiography shows that dermal lipogenesis is localized mainly in pilo-sebaceous units (Cooper *et al.*, 1974, and unpublished results). There is evidence from animal studies of the importance of glucose as a substrate for lipogenesis in skin (Wheatley *et al.*, 1971; Wheatley, 1974), and it is likely that under the conditions used here, glucose incorporation parallels the absolute rate of lipogenesis.

Although the correlation found in this study between dermal lipogenesis and severity of acne (Fig. 1) was not statistically significant, this is probably an effect of the sample size (n = 20). Studies which have shown a significant correlation between SER and severity of acne have involved series numbering from 52 upwards (Pochi & Strauss, 1964; Cunliffe & Shuster, 1969a; Burton & Shuster, 1971; Cotterill *et al.*, 1971), whereas smaller series have not yielded significant correlations (Fry & Ramsay, 1966; Powell & Beveridge, 1970). Further studies of dermal lipogenesis will therefore be necessary to confirm the relationship with severity of acne that is indicated by present results.

At high rates of lipogenesis, the percentage labelling of neutral lipids in the dermis tended to increase. This could mean that as sebaceous activity increases, more neutral lipid is produced for 'export' relative to the polar lipids needed for structural purposes. However, it is likely that some, at least, of the effect seen is due to the presence of polar lipid labelling in follicles, which would dilute out any increase in polar lipid labelling in the sebaceous glands.

To examine the relative proportions of the major secretory lipids, independently of changes in polar lipid percentage, we calculated the ratio of wax esters to triglyceride and squalene, and that of squalene to triglyceride. These calculations brought to light a relative increase in wax ester labelling at high lipogenic rates, but this effect was quantitatively quite small. More surprising was the failure to detect any significant increase in squalene labelling relative to other lipids, either with increasing lipogenic rate (Table 1) or in severe acne (Fig. 4a). This appears to conflict with Summerly's finding (1974) of an increased percentage labelling of squalene in sebaceous glands harvested from acne-prone

TABLE 3. Comparison of surface lipid composition (back) and pattern of sebaceous lipogenesis

	Free fatty acid (%)	Sterol (%)	Diglycerides (%)	Triglycerides (%)	Wax esters + sterol esters (%)	Squalene (%)
Surface lipid						
Haahti (1961) (n = 2)	16·2	1·4	7·1	33·4	24·2	15·6
Lewis & Hayward (1971) (n = 23)	16·0	1·8		46·4*	24·4	11·4
Lipogenesis						
Summerly (1974) (n = 10)	2·3		3·9†	51·7	19·8	22·3
Present results (n = 20)	3·0	1·1	5·5	54·7	11·1	23·8

* Includes mono- and diglycerides; † Includes sterols.

Surface lipid results are gravimetric determinations following column chromatography of samples from males aged 24–30 years (Haahti, 1961) or 17–23 years (Lewis & Hayward, 1971). Lipogenesis results are for isolated sebaceous glands from control subjects of both sexes in the acne age range (Summerly, 1974), or total dermal lipogenesis in biopsies from males aged 16–26 years (present results). For comparison, percentages have been recalculated where necessary to exclude polar lipids, monoglycerides and saturated hydrocarbons.

skin; further work will be necessary to elucidate the reasons for this. Otherwise, our results for the pattern of lipogenesis in the acne age group agree quite closely with those of Summerly (1974), and show several differences from the composition of surface lipid from the back (Table 3). Apart from the marked difference in the proportion of free fatty acid, which may reflect esterase activity beyond the sebaceous gland, both labelling studies show a greater percentage of squalene and a smaller percentage of wax esters plus sterol esters than are found in surface lipid. There are several possible explanations for this, including failure to attain uniform labelling throughout the sebaceous gland, removal of squalene from the secreted lipid subsequent to synthesis, or even a circadian rhythm in the synthesis of sebaceous components (Downing, 1974). This finding would merit further study.

The present results also show a relationship between age and sebaceous lipogenesis. With increasing age there was a decrease in the synthesis of squalene, relative to both wax esters and triglyceride (Table 2 and Fig. 4a). Wax ester labelling also declined relative to triglyceride. These findings are consistent with the observation of Cotterill *et al.* (1972) that the percentages of squalene and wax esters in surface lipid are higher in males in the 26–40 years age group than in the 41–60 years age group. Both squalene and wax esters are said to be comedogenic (Kligman, Wheatley & Mills, 1970), but we think it premature to conclude that the decline in synthesis of these lipids with age is a factor in the clinical improvement of acne which occurs eventually and in spite of continuing seborrhoea (Cunliffe & Shuster, 1969a; Cotterill *et al.*, 1972).

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REFERENCES

Boughton, B., Mackenna, R.M.B., Wheatley, V.R. & Wormall, A. (1959) The fatty acid composition of skin surface fats (sebum) in acne vulgaris and seborrhoeic dermatitis. *Journal of Investigative Dermatology*, **33**, 57.

Burton, J.L. & Shuster, S. (1971) The relationship between seborrhoea and acne vulgaris. *British Journal of Dermatology*, **84**, 600.

Cooper, M.F., McGrath, H. & Shuster, S. (1976) Epidermal lipogenesis in psoriasis and lichen simplex. *British Journal of Dermatology*, in press.

Cooper, M.F., Thody, A.J. & Shuster, S. (1974) Hormonal regulation of cutaneous lipogenesis; effects of hypophysectomy, posterior-hypophysectomy and α-melanocyte-stimulating hormone treatment. *Biochimica et biophysica Acta*, **360**, 193.

Cotterill, J.A., Cunliffe, W.J. & Williamson, B. (1971) Severity of acne and sebum excretion rate. *British Journal of Dermatology*, **85**, 93.

Cotterill, J.A., Cunliffe, W.J., Williamson, B. & Bulusu, L. (1972) Further observations on the pathogenesis of acne. *British Medical Journal*, **iii**, 444.

Cunliffe, W.J. & Shuster, S. (1969a) Pathogenesis of acne. *Lancet*, **i**, 685.

Cunliffe, W.J. & Shuster, S. (1969b) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697.

Downing, D.T. (1974) Synthesis and composition of surface lipids of human skin. *Journal of Investigative Dermatology*, **62**, 228.

Fry, L. & Ramsay, G.A. (1966) Tetracycline in acne vulgaris. Clinical evaluation and the effect on sebum production. *British Journal of Dermatology*, **78**, 653.

Fulton, J.E., Anderson, A. & Randall, S. (1971) Sebum excretion: analysis by infra-red spectroscopy. *Journal of Investigative Dermatology*, **56**, 413.

Gloor, M., Graumann, U., Kionke, M., Wiegand, I. & Friederich, H.C. (1972) Skin surface lipids. 1. Qualitative and quantitative determination in patients with acne vulgaris and controls. *Archiv für Dermatologische Forschung*, **242**, 316.

Haahti, E. (1961) Major lipid constituents of human skin surface. *Scandinavian Journal of Clinical and Laboratory Investigation*, **13**, Supplement 59.

Kellum, R.E. & Strangfeld, K. (1972) Acne vulgaris: studies in pathogenesis. Fatty acids of human surface triglycerides from patients with and without acne. *Journal of Investigative Dermatology*, **58**, 315.

Kligman, A.M., Wheatley, V.R. & Mills, O.H. (1970) Comedogenicity of human sebum. *Archives of Dermatology*, **102**, 267.

Krakow, R., Downing, D.T., Strauss, J.S. & Pochi, P.E. (1973) Identification of a fatty acid in human skin surface lipids apparently associated with acne vulgaris. *Journal of Investigative Dermatology*, **61**, 286.

Lewis, C.A. & Hayward, B.J. (1971) Human skin surface lipids. In: *Modern Trends in Dermatology*, vol. 4. Butterworths, London.

Pochi, P.E. & Strauss, J.S. (1964) Sebum production, casual sebum levels, titratable acidity of sebum, and urinary fractional 17-keto-steroid excretion in males with acne. *Journal of Investigative Dermatology*, **43**, 383.

Powell, E.W. & Beveridge, G.W. (1970) Sebum excretion and sebum composition in adolescent men with and without acne vulgaris. *British Journal of Dermatology*, **82**, 243.

Runkel, R.A., Wurster, D.E. & Cooper, G.A. (1969) Investigation of normal and acne skin surface lipids. *Journal of Pharmacological Science*, **58**, 582.

Salamon, T., Bobareuic, B., Lazovic, O., Pujic, Z. & Hadzimusic, M. (1970) The relationship of esters and free fatty acids in the skin surface lipids in persons with some dermatoses. *Acta dermato-venereologica*, **50**, 176.

Siegel, S. (1956) *Nonparametric Statistics for the Behavioural Sciences*. McGraw-Hill, New York.

Summerly, R. (1974) Skin components: ^{14}C acetate incorporation *in vitro*. *The Transactions of St. John's Hospital Dermatological Society*, **60**, 1.

Wheatley, V.R. (1974) Cutaneous lipogenesis. Major pathways of carbon flow and possible interrelationships between the epidermis and sebaceous glands. *Journal of Investigative Dermatology*, **62**, 245.

Wheatley, V.R., Hodgins, L.T., Coon, W.M., Kumarasiri, M., Berenzweig, H. & Feinstein, J.M. (1971) Cutaneous lipogenesis: precursors utilised by guinea-pig skin for lipid synthesis. *Journal of Lipid Research*, **12**, 347.

British Journal of Dermatology (1976) 94, 173.

Lymphocyte abnormalities in mycosis fungoides

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SUMMARY

Fourteen patients with clinical and histological evidence of mycosis fungoides have undergone a series of immunological tests. Significant findings include the presence of low numbers of E and EAC rosette-forming cells in the peripheral circulation of the mycosis fungoides patients. Elevated levels of IgE were seen in five of the mycosis fungoides patients, and the mean IgE level was significantly higher than in the control series. Possible explanations of these findings are considered.

Sequential clinical observations on patients with histologically proven mycosis fungoides (MF) illustrate clearly and at times dramatically the very varied clinical course which may ensue. A small proportion of patients present with cutaneous nodules or tumours, and proceed rapidly to death from intercurrent infection; more common, however, is the insidious development of pruritus, plaques, and possibly erythroderma over a period of years or even decades, with the eventual cause of death being apparently unrelated to the dermatological disorder.

Such a spectrum of disease activity strongly suggests that in MF host resistance may play a part in determining the progression of the disease, and for this reason we have examined a group of patients with this disorder with the aim of identifying immunological abnormalities which may prove to be of prognostic value in assessing these patients.

PATIENTS

Details of age, sex, clinical appearance and duration of MF at the time of study are given in Table 1. Patients selected for study were those who had histologically proven MF, using Lever's criteria (Lever, 1967). This includes pleomorphism and deep invasion of the dermal infiltrate, the presence of mitotic figures within this infiltrate, and the presence of Pautrier abscesses within the epidermis. All patients were alive at the end of the study, with cutaneous lesions relatively well controlled.

METHODS

Investigations carried out included total lymphocyte count, quantification of percentages of circulating T and B lymphocytes by rosetting techniques and also of absolute numbers of circulating T and B lymphocytes, quantification of levels of circulating IgG, A, M, D, and IgE, direct immunofluorescence of lesional skin using fluorescein-conjugated antisera to these immunoglobulins, lymphocyte transformation on exposure to phytohaemagglutinin (PHA) and pokeweed mitogen (PWM), and skin

TABLE 1. Mycosis fungoides patients. Details of age, sex, duration of lesions, and clinical state at the time of study

Patient no.	Age	Sex	Duration of lesions	Clinical appearance
1	37	M	3 months	Erythroderma
2	61	F	10 months	Poikiloderma and plaques
3	76	F	1 year	Elevated plaques
4	54	F	11 years	Tumours and poikiloderma
5	48	M	2 years	Plaques
6	44	M	3 years	Plaques
7	49	F	5 years	Arcuate raised lesions
8	74	M	20 years	Gross poikiloderma
9	36	F	1 year	Plaques
10	77	M	14 months	Plaques
11	38	M	3 years	Plaques and acquired ichthyosis
12	73	M	4 years	Plaques
13	76	M	2 years	Exfoliative erythroderma
14	63	F	20 years	Plaques

testing to recall antigens PPD (1 in 100), Candida and *T. rubrum* (Bencard), mumps (Eli Lily) and streptokinase–streptodornase (Lederle).

Rosetting technique

Lymphocytes were prepared from heparinized whole blood by separation on a Ficoll-Triosil mixture (SG 1.077). After two washes they were re-suspended in Eagles medium at a concentration of 4×10^6/ml. For T lymphocyte identification the E rosette technique of Jondal, Holm & Wigsell (1972) was used; 0·25 ml aliquots of lymphocyte suspension were incubated for 15–20 min at 37°C with an equal volume of 1% washed sheep red blood cells, pelleted and incubated for 24 h at 4°C. The cells were then gently re-suspended and the percentage of rosette-forming cells estimated. A minimum of 400 lymphocytes were counted. For the estimation of EAC rosettes (B lymphocytes) 0·25 ml aliquots of a 1% suspension of sheep red blood cells which had been freshly coated with rabbit anti-sheep red blood cell haemolysin and normal human plasma as a source of complement were pelleted together with an equal volume of the lymphocyte suspension. The cell pellets were incubated together at 37°C for 15–20 minutes, re-suspended and the number of rosette-forming lymphocytes counted (Bianco, Patrick & Nussenzweig, 1970).

It is appreciated that the EAC technique will in addition identify any remaining monocytes in the lymphocyte-rich preparations, but studies in our laboratory indicate that such contamination is less than 5% of total cells recovered after Ficoll-Triosil separation.

Immunoglobulins IgG, A, M and D were quantified by single radial immunodiffusion using Hoechst plates and immunoglobulin standard, and IgE by radio-immunoassay.

Direct immunofluorescence of involved skin was carried out using IgG, A and M from Hyland Laboratories and IgD and E from Hoechst. All were used at a dilution of 1 in 32. Frozen sections were cut at 5–7 μm, incubated with the conjugates for 30 min, washed 3 times in phosphate-buffered saline and mounted in buffered glycerol. They were then examined in a Leitz orthomat orthoplan fluorescent microscope lit by a high pressure mercury vapour lamp using a BG 38 filter, FITC3 interference primary filter and a K510 secondary filter.

For lymphocyte transformation 2×10^5 lymphocytes were incubated in Eagles medium in a moist 37°C chamber with 5% CO_2. PHA (Wellcome) was used at a final dilution of 1 in 10, and PWM (Grand Island Biological Co.) at a dilution of 1 in 5. Cultures were incubated for 72 h and ^{14}C-

TABLE 2. T and B cells in peripheral circulation

Patient no.	Absolute lymphocyte count/mm^3	% T cells	Absolute T cells/mm^3	% B cells	Absolute B cells/mm^3	% null cells	Absolute null cells/mm^3
1	1881	43	808	38	714	19	357
2	7320	40	2928	9	66	51	3730
3	1696	60	1020	51	846	—	
4	1170	50	585	53	620	—	
5	1474	60	844	31	456	9	133
6	1800	52	936	45	810	3	54
7	1001	50	500	40	400	10	100
8	1872	57	1107	34	635	9	168
9	1776	48	852	52	923	—	
10	2912	61	1800	28	815	11	320
11	1650	48	792	40	660	12	198
12	1445	36	520	39	563	25	361
13	2210	70	1047	20	440	10	221
14	1848	64	1232	32	616	2	37
Mean ± s.d.	2146 ± 1556	52 ± 9·6	1105 ± 641	36 ± 12·4	611 ± 222	11 ± 13	149 ± 134
Control mean ± s.d.	2580 ± 405	68 ± 3·1	1705 ± 76	31 ± 4·1	781 ± 109	1·4 ± 2·4	33 ± 54
	ns	$P<0{\cdot}0001$	$P<0{\cdot}0001$	ns	$P<0{\cdot}006$	$P<0{\cdot}003$	$P<0{\cdot}003$

thymidine added for the last 4 h (0·2 μCi per well). After extraction of cell suspensions with 10% trichloracetic acid and 2 ml absolute alcohol, the filter paper was dried in air. Radioactivity was measured in a Packard tricarb scintillation counter and expressed as counts per min per 10^6 cells.

The patients used as controls in this study were age and sex matched, and had no dermatological disorder. The majority were patients admitted for minor surgical procedures and all were tested prior to anaesthesia.

RESULTS

Total lymphocyte counts, percentages, and absolute values of circulating T and B lymphocytes are given in Table 2. Also included are levels of 'null cells', cells forming rosettes by neither method. It will be seen that, despite a normal mean total lymphocyte count for the MF group, they have a highly significant decreased percentage of circulating T lymphocytes ($P<0·0001$) and also a highly significant decrease in absolute levels of T lymphocytes per mm^3 of peripheral blood ($P<0·0001$). In contrast, the percentage of circulating B cells is not significantly different in the MF group, but the absolute level of B lymphocytes is highly significantly lower than in the control group ($P<0·006$). The percentage of null cells is significantly increased in the MF group as are the absolute numbers of null cells.

Immunoglobulin levels are recorded in Table 3, together with details of immunoglobulins detected by direct immunofluorescence of lesional skin. The mean levels of IgG, A, M and IgD do not differ significantly from control values, although there are two patients with elevated IgA levels (3 and 10) and two with elevated IgD (6 and 7). The IgE levels, however, are raised in five patients (4, 5, 7, 12 and 14) and the mean IgE level of the group is significantly higher than the control mean ($P<0·001$). Small aggregates of immunoglobulin were detected in the dermis in ten patients, but it will be seen that there is no correlation between the predominant class identified and circulating immunoglobulin levels of these classes. In two patients (8 and 14) epidermal deposits of immunoglobulin were also identified; both these patients had striking poikiloderma.

TABLE 3. Circulating immunoglobulin levels (international units per ml) and immunoglobulins detected by immunofluorescence in the dermis

Patient no.	IgG	IgA	IgM	IgD	IgE	Dermal Ig deposits
1	216	256	96	25	156	IgG
2	100	64	208	0	60	IgG
3	138	810	142	0	68	IgM
4	89	118	87	0	488	IgA+M
5	112	196	113	19	1280	None
6	205	240	112	135	84	None
7	160	221	222	53	1480	IgD
8	96	220	140	16	165	IgA+M
9	151	190	226	10	146	IgG
10	210	340	112	0	400	IgE
11	138	154	94	24	456	None
12	205	112	86	18	163	IgD
13	104	126	118	0	84	IgG
14	195	190	120	18	6000	None
Mean ± s.d.	151±47	226±182	134±48	21·1±35	402±451	
Control mean ± s.d.	147±45 ns	179±54 ns	135±47 ns	24±7·2 ns	91±53 $P<0·001$	

Mitogen mediated lymphocyte transformation results are detailed in Table 4. All patients responded reasonably well to both mitogens, but in two patients (1 and 6) the response to PWM was better than that to PHA, resulting in a reverse of the normal ratio when counts per min per 10^6 lymphocytes in response to PHA and PWM are compared. Normally in our laboratory this figure is 2 or higher, and there may be therefore a small deficit in PHA transformation.

TABLE 4. Lymphocyte transformation measured by ^{14}C thymidine incorporation into DNA at 72 h (counts/min/10^6 lymphocytes)

Patient no.	Control culture (no mitogen)	PHA	PWM	Ratio PHA: PWM
1	192	7853	7991	0·98
2	179	3516	1334	2·6
3	150	1910	1465	1·3
4	658	7967	3874	2·1
5	1497	11,210	5259	2·1
6	46	3125	3340	0·93
7	1012	10,160	4053	2·5
8	178	11,204	6327	1·77
9	142	9374	3929	2·38
10	127	8787	8692	1·0
11	350	7510	4462	1·7
12	36	2651	1150	2·3
13	48	1437	847	1·7
14	12,339	1,272,490	937,528	1·35

Skin testing. No topical steroid was applied to the sites tested for 2 weeks prior to the tests. Results were read at 48 h and all five tests applied to each of the fourteen MF patients. Of seventy tests applied twenty-six (37%) were positive compared with thirty-six (51%) in the control group. Only two MF patients (1 and 2) demonstrated total intradermal anergy by reacting to none of the applied antigens.

DISCUSSION

The striking findings in this study lie in the differences between the MF and control group in the numbers of circulating E and EAC rosette-forming cells. If these are taken as representing T and B lymphocytes respectively, then the MF patients have significantly reduced levels of circulating T and B cells, and a high level of null lymphocytes possessing neither marker. It is of note that these abnormalities are present despite a normal mean total lymphocyte count. One of the few reports of patients with MF having an absolute lymphopenia comes from Fuks, Bagshaw & Farber (1973), who reported 21% of a series having less than 1000 lymphocytes per ml, and an associated poor prognosis for patients in this category. No patient in this present series had a lymphopenia of this degree.

Where then are the E and EAC rosette-forming cells in the MF patients? There would appear to be three possibilities; firstly, that these patients have always had low levels of rosette forming cells, and that the development of MF is a consequence of this immune deficiency, rather than a result of the disease process. Preliminary observations on a small number of patients with very early premycotic lesions indicate that some of these patients also have low levels of rosette-forming cells, so that this would appear to be a distinct possibility. Secondly, there is the possibility that the rosette-forming lymphocytes are not present in the peripheral circulation, because they have moved to another body compartment, in this case the skin, drawn there either by changes in the dermis or epidermis or by changes affecting the lymphocytes themselves and their natural circulation pathways. This sort of maldistribution could be initiated by such external events as a viral infection. Thirdly, there is the possibility of rapid destruction of rosette-forming cells. This is a difficult theory to investigate *in vivo* in view of the longevity of the T lymphocyte, but there is no evidence of inadequate function of B lymphocytes in the form of low levels of any of the immunoglobulins measured.

The elevated levels of IgE reported in this study confirm those reported by Tan *et al.* (1974). None of the patients in our series with elevated IgE levels had either evidence or a history of atopy. They did not appear to form a subgroup in terms of clinical features or of duration of disease. It is intended to use RAST tests for specific allergens to identify the nature of the IgE in these patients. The significance of raised IgE levels in MF, and their relationship, if any, to the lymphocyte abnormality, is not at present clear. This finding, together with the recent observations of Arbesman *et al.* (1974) of elevated IgE levels in bullous pemphigoid, suggest that IgE may well be of importance in dermatoses other than atopic dermatitis.

At the present time direct immunofluorescence of involved skin appears to offer little additional information in MF, and it is anticipated that the identification of rosette-forming cells in skin sections may be more fruitful.

The relatively normal responses in the lymphocyte transformation studies confirm findings previously reported by other workers. It is of note that even in patients with rosette-forming cells totalling less than 50% of the circulating lymphocyte population, normal levels of transformation can be obtained. This would suggest that the use of this test in situations where subtle variations in the lymphocyte population are present, rather than an absolute deficiency, may be of doubtful value.

The low response rate to intradermal skin tests is difficult to interpret. Although all patients tested refrained from applying topical steroids to the area for 2 weeks prior to skin testing there may still have been a reservoir of steroid present.

These observations indicate that in MF there are abnormalities of the circulating lymphocyte population; the relationship of these abnormalities to the timing of disease progress clearly requires further study. Already a reticulosis register such as that established by Dr Peter Samman has established its value in demonstrating the clinical progress of the premycotic eruptions, and it is hoped that as new immunological techniques become available they will be applied to patients in such registers to further our understanding of the natural progress of MF, its associated immunological abnormalities, and their modification by treatment.

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REFERENCES

ARBESMAN, C.E., WYPYCH, J.I., REISMAN, R.E. & PEUTNER, E.M. (1974) IgE levels in sera of patients with pemphigus or bullous pemphigoid. *Archives of Dermatology*, **110**, 378.

BIANCO, C., PATRICK, R., & NUSSENZWEIG, V. (1970) Population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. *Journal of Experimental Medicine*, **132**, 702.

FUKS, Z.Y., BAGSHAW, M.A. & FARBER, E.M. (1973) Prognostic signs and the management of mycosis fungoides. *Cancer*, **32**, 1385.

JONDAL, M., HOLM, G. & WIGSELL, H. (1972) Surface markers on human T and B lymphocytes. *Journal of Experimental Medicine*, **136**, 207.

LEVER, W.F. (1967) *Histopathology of the Skin*, 4th edn. Pitman Medical, London.

TAN, R.S.H., BUTTERWORTH, C.M., MCLAUGHLIN, H., MALKA, S. & SAMMAN, P.D. (1974) Mycosis fungoides—a disease of antigen persistence. *British Journal of Dermatology*, **91**, 607.

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International mortality from bullous diseases since 1950

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SUMMARY

National mortality figures hold information about our overall success in treating bullous disorders which cannot be found elsewhere. The official data, since 1950, for England and Wales, the United States, France, Japan, Scotland, Ireland and Denmark have been analysed after corrections for changes in the population structure and in the International Classification of Disease.

A steep fall in deaths occurred in England and Wales between 1952 (121 deaths) and 1955 (55 deaths). The low rate persisted from 1955 to 1962 (40 deaths) when it fell to a steady even lower level lasting until the present. The United States figures showed a sharp drop in mortality starting about 2 years earlier (from 283 deaths in 1950 to 150 deaths in 1954), followed by a gradual fall to the present. Mortality in Scotland, Ireland and Denmark has followed the same general pattern but the figures from France, after a steady fall between 1951 (68 deaths) and 1965 (29 deaths), have risen again until 1971 (62 deaths). The number of deaths has not fallen in Japan but the data are difficult to interpret.

The timing of the improvement in mortality in England and Wales, and in the United States, fits well with the introduction of systemic steroids into general use. A fall to about one-third of the pre-steroid level is in general agreement with the improvement recorded in smaller personal series from several centres.

An epidemiological approach to dermatology has sometimes proved to be rather unrewarding. Incidence figures suffer from variable, and often inadequate, recording systems, and because many patients with trivial skin complaints may never attend their doctors. These objections do not apply to an incident as dramatic as death from a bullous disease, and national mortality figures hold information about our overall success in treating bullous diseases which cannot be found in any other way. This paper is an analysis of the official figures for death from chronic bullous diseases since 1950. The details for England and Wales are presented fully and are compared with data from the United States France, Scotland, Ireland, Denmark and Japan.

Sources of information

The Registrar General's Office has published statistical reviews for England and Wales each year since 1839. These volumes have become steadily larger and more complex but not necessarily more accurate (*British Medical Journal*, 1971). They deal with a variety of matters including changes in the structure of the population, and mortality from different diseases. Terminology has followed the

International Classification of Disease which was originally based on a classification prepared in 1855 by William Farr and later on a classification devised by Jacques Bertillon in 1893. It is revised at approximately 10-year intervals. The 6th review of the classification, in 1948, was incorporated into the annual statistics review in 1950. The 7th review was incorporated in 1957 and the 8th in 1968. Information about England and Wales has been drawn largely from these statistical reviews, and from correspondence with the Office of Population Censuses and Surveys.

Publications comparable to the *Registrar General's Annual Statistical Reviews* have been consulted for the United States (*Vital Statistics of the United States*), France (*Statistiques des causes médicales de décès*), Ireland (*Report on Vital Statistics*), Scotland (*Annual Report of the Registrar-General for Scotland*), Denmark (*Causes of Death in the Kingdom of Denmark*), and Japan (*Vital Statistics of Japan*).

Diagnostic categories

The changing use of diagnostic categories has been a major obstacle to overcome in arriving at an estimate of deaths from pemphigus, pemphigoid, and dermatitis herpetiformis. For simplicity the changes in the Registrar General's Annual Reviews since 1940 are shown in Table 1. Since its 6th review, in 1948, the International Classification of Diseases has used a 4-figure system which separates dermatitis herpetiformis (category 704·0) from pemphigus (704·1) The *Annual Statistical Review for England and Wales*, however, has used only the first three figures; and so dermatitis herpetiformis and pemphigus (both category 704) were not separated until the 8th revision of the classification (1965) was incorporated into the *Annual Review* for 1968. Dermatitis herpetiformis then fell into the category 693 and pemphigus cases into 694. This separation between dermatitis herpetiformis and pemphigus was also made in 1968 in the figures from the United States, France, Ireland, Japan and Scotland and in the Danish figures in 1969.

TABLE 1. The altering diagnostic categories used in the Registrar General's Annual Statistical Reviews for England and Wales

	1938–49	1950–57	1958–67	1968–present
Dermatitis herpetiformis	153	704	704	693
Pemphigus	153	704	704	694
Pemphigoid	—	—	704	694
Erythema multiforme	153	705	705	695

Deaths in England and Wales

The data in Fig. 1 have been obtained from the following diagnostic categories: 1950 to 1967—category 704; 1968 to 1972—categories 693 and 694. Dermatitis herpetiformis, as explained above, was in the same category as pemphigus until 1968, and so the figures up to that time would have included deaths from both diseases. It is for this reason, to keep the figures comparable, that the data from which Fig. 1 was drawn have from 1968 onwards included both category 693 (dermatitis herpetiformis) and 694 (pemphigus and pemphigoid).

Pemphigoid was first established as an entity, separate from the other bullous disorders, in the early 1950s (Lever, 1953; Rook & Waddington, 1953). Before that, cases were likely to have been classed as pemphigus, as dermatitis herpetiformis, or as erythema multiforme. Pemphigus and dermatitis herpetiformis were already in the same category, so reclassifying these earlier cases would make no difference to the overall figures. However, it is possible that some deaths which would formerly have been classified as due to erythema multiforme might have been included in the pemphigus–dermatitis herpetiformis group after 1953. In fact, at that point, there was no gain at all in this group. The figures for erythema multiforme itself are hidden in a mass of other 'erythematous conditions' but there was no sharp fall in this general category either. It cannot be profitable, at this stage, to try to unravel the diagnostic tangle further.

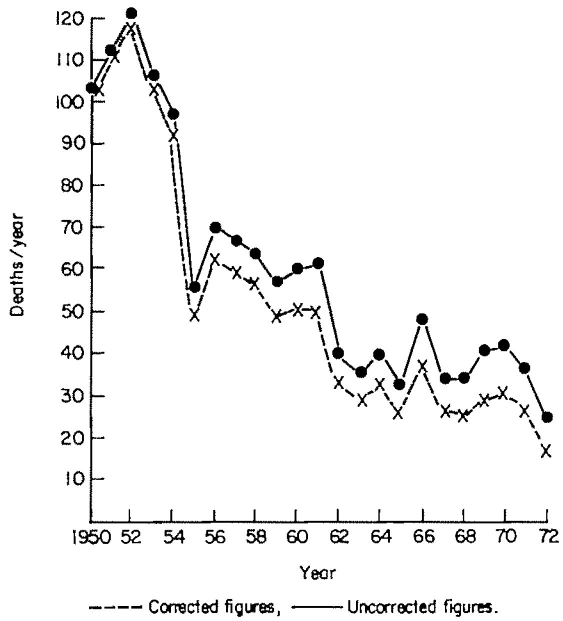

FIGURE 1. Deaths from bullous diseases in England and Wales between 1950 and 1972: absolute figures and figures corrected for changes in population structure.

Fig. 1, therefore, with these provisos, represents deaths from the three major bullous diseases: pemphigus, pemphigoid and dermatitis herpetiformis. Of these, dermatitis herpetiformis is unlikely to have contributed many deaths. It is recognized as benign, and treatment with arsenic was effective even before sulphapyridine was introduced (Barling, 1944); the more recent use of dapsone in this country (Calnan, 1954) has been an alternative rather than a superior form of treatment. The figures for dermatitis herpetiformis as a separate diagnostic category are known from 1959 onwards, and have averaged only 3·0 deaths per year. The great bulk of deaths shown in Fig. 1 will, therefore, have been from pemphigus and pemphigoid.

The most arresting feature of Fig. 1 is the steep drop in deaths between 1952 (121 deaths) and 1955 (55 deaths), and particularly between 1954 (97 deaths) and 1955. The new low rate persisted from 1955 to 1962 (40 deaths), when it fell to a steady but even lower level, lasting until the present. It follows from what has been said that these changes were not due simply to alterations in the diagnostic categories. The 6th and 7th revisions of the International Classification of Disease did not differ in the categories used for bullous diseases, and so the categories in the *Annual Statistical Reviews* were unchanged between 1950 and 1967—the period when the major fall in deaths was seen. The introduction of the term pemphigoid in 1953 would not have affected the numbers except perhaps to attract into category 704 those cases which previously would have been included in the erythema multiforme group. The observed fall in deaths cannot be explained in this way. Nor has there been any suggestion that the incidence of bullous diseases has fallen during the last 25 years, although this is difficult to prove. Certainly no fewer cases presented at St John's Hospital for Diseases of the Skin (Neves, 1966) or at skin clinics in Northern Ireland since 1962 (Burrows, D., personal communication).

There have been major changes in the population at risk in England and Wales during this period: in 1950 the total population was 43,830,000 and by 1972 this had risen by 12·0% to 49,101,000. Both pemphigus (average age at death 65 years) and particularly pemphigoid (average age at death 75 years) (Savin, unpublished observations) tend to affect older people. Between 1950 and 1972, the population over 50 years old had increased by 29·25% (from 11,777,000 to 15,222,000) and the number

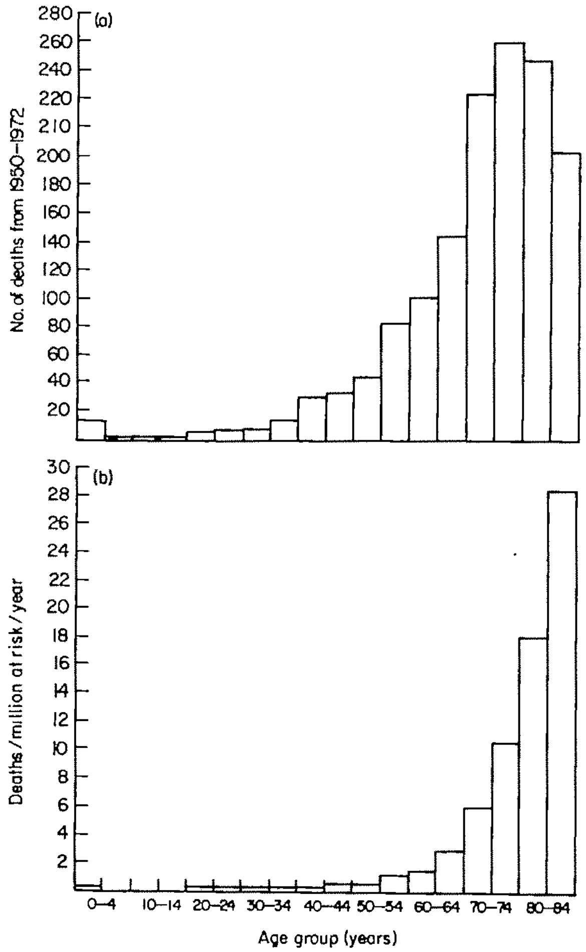

FIGURE 2a. Deaths from bullous diseases in England and Wales between 1950 and 1972 classified by age at death.

FIGURE 2b. Deaths from bullous diseases in England and Wales from 1955 to 1972, calculated per million people in each age group at risk per year.

over 70 years had risen by 37·6% (from 2,975,000 to 4,092,000). Thus the older sections, those at risk for pemphigus and pemphigoid, have proportionately risen the most quickly.

This change should have raised rather than lowered the numbers dying from bullous disease. Fig. 1 also shows the mortality data directly standardized to take into account the changing age and sex structure of the population (Armitage, 1971). The sudden and persistent lowering of the death rate in the early 1950s becomes slightly more obvious in this way: it runs counter to the rise in that part of the population which is particularly at risk.

When considering all deaths from bullous diseases from 1950 to 1972 (Fig. 2a), the peak can be seen to lie in the 75–79 year age-group. However, when corrected to take into account the size of the population in each age group, it becomes clear that the risk of an individual dying from bullous disease is highest for those over 85 (Fig. 2b). This is particularly striking in males whose risk of dying

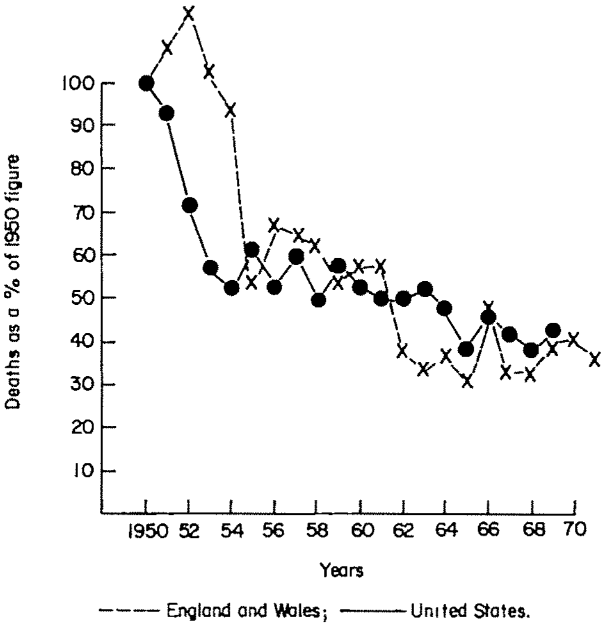

FIGURE 3. Deaths from bullous diseases in the United States, and in England and Wales from 1950 to 1972. Figures calculated as a percentage of the 1950 figure.

from a bullous disease over the age of 80 is roughly twice that of females. No doubt this relates partly to the especially high incidence of pemphigoid in elderly males which has already been documented (Lim, MacDonald & Rook, 1968); however, the death rate from all causes is higher in men than in women of this age group. The improvement in mortality after 1954 is very obvious for all age groups, but particularly so in the younger ones which are likely to have contained a high proportion of pemphigus cases.

The official census in Britain does not include an enquiry about religious affiliation and it is therefore hard to estimate changes in sub-groups of the population which are unusually susceptible to bullous diseases. The Jewish population, with its excess of pemphigus, can only be counted indirectly, for example by collecting data on burials and cremations, and by assuming that the mortality rate of the Jewish population is comparable to that of the general population (Prais & Schmool, 1968). However, this is unreliable as Jewish mortality is between 5 and 15% lower than general mortality

(Prais, 1967) and so a correction factor has to be introduced into the calculations. The Jewish population of Great Britain is estimated to have increased by 21·7% between 1935 (337,000) and 1968 (410,000). This increase is close to the 19·6% recorded for the total population of England and Wales over the same period. It represents an absolute gain in the Jewish population and cannot account for the actual fall in deaths from chronic bullous diseases.

Mortality in other countries

The United States of America. The figures for the United States are shown in Fig. 3, expressed as a percentage of the 1950 figures for comparison with England and Wales. The fall in deaths has been just as dramatic, but took place about 2 years earlier (from 283 deaths in 1950 to 150 deaths in 1954).

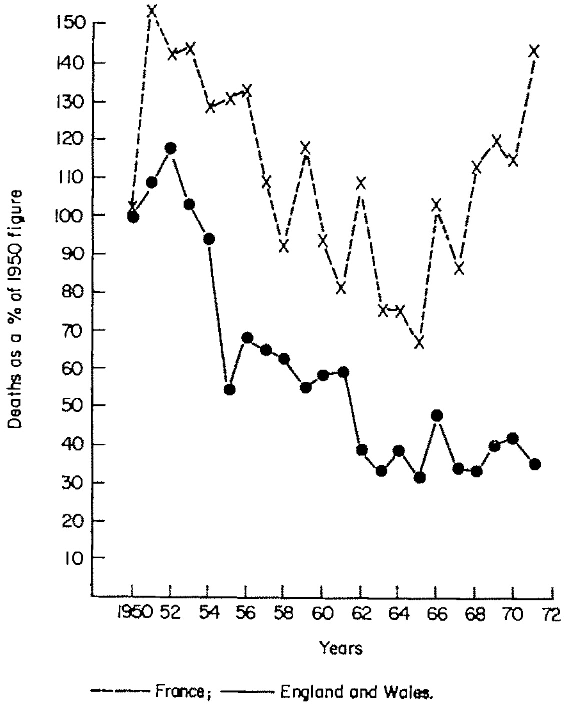

FIGURE 4. Deaths from bullous diseases from 1950 in France (principal cause of death) and in England and Wales. Figures expressed as a percentage of the 1950 figure.

Between 1954 and 1969 (123 deaths), there was only a very slow fall. The population over 65 has risen even more rapidly in the United States than in England and Wales and correcting for this makes the improved mortality even more obvious.

France. The French graph is quite different, with a slow fall between 1951 (68 deaths) and 1965 (29 deaths), but then showing an upswing to 1971 (62 deaths) (Fig. 4). This cannot be explained in terms of an ageing population—the increase over 65 has been equivalent to that of England and Wales. However, it is clear that the diagnostic categories have been used differently in France. Since dermatitis herpetiformis has been separated from pemphigus in the French figures (1968–1971) there have been more deaths from dermatitis herpetiformis (119 deaths) than from pemphigus (94 deaths). This is

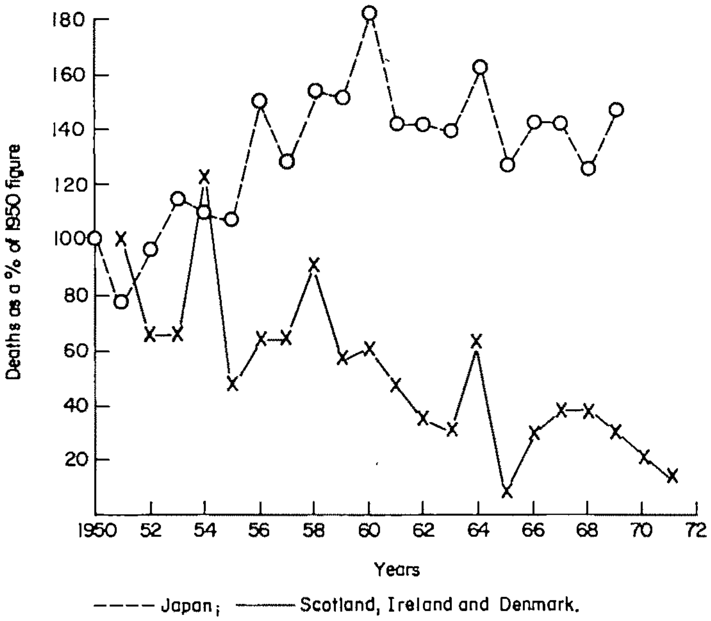

FIGURE 5. Deaths from bullous diseases in Japan, and in Denmark, Ireland and Scotland combined. Figures expressed as a percentage of the 1950 figure.

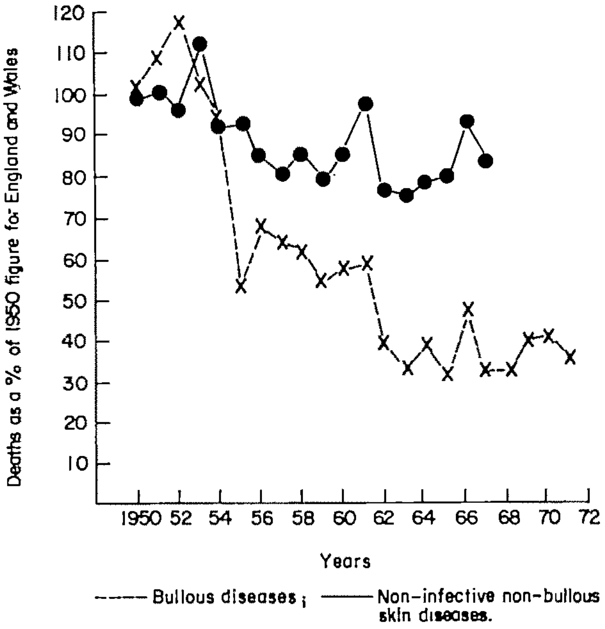

FIGURE 6. Deaths from bullous diseases in England and Wales contrasted with deaths from non-bullous, non-infective skin diseases.

very different from the proportion in the United States (67 deaths from dermatitis herpetiformis and 1546 from pemphigus and pemphigoid between 1958 and 1969) and in England and Wales (47 and 579 respectively between 1958 and 1971). The validity of separating pemphigoid from dermatitis herpetiformis has been less well accepted in France than in other countries. Recent evidence derived from immunofluorescence studies has made the distinction sharper but no doubt some cases which in England and the United States would have been classed as pemphigoid have, in France, been included as dermatitis herpetiformis. However, the figures for dermatitis herpetiformis (category 693) and pemphigus/pemphigoid (category 694) have been pooled in Fig. 5 and the detailed split between them should not affect the issue.

Denmark, Ireland and Scotland. The figures for these countries have been treated together, perhaps unjustifiably, to give a combined population at risk of about 13 million. Subject to the wider fluctuations to be expected from such a small population, mortality has fallen in a way comparable to that of England and Wales and the United States (Fig. 5).

Japan. The results here are much more difficult to interpret. In 1950, for example, of the 75 deaths recorded in category 704, 27 were those of children under 5 years old. This age group contributed substantially to category 704 until 1960. Death from bullous disorders is rare at this age in other countries, and possibly cases with an infectious cause, such as pemphigus neonatorum, may have been included. In Fig. 5, deaths under the age of 5 years have been excluded but this should not account for the apparent increase in deaths. The older age groups have increased very markedly in Japan, and this, perhaps with alterations in certification, may have played some part. Further speculation is useless without personal knowledge of the changes in medical practice in that country.

TABLE 2. Dates of first mention of steroids in British pharmaceutical publications

Substance	British Pharmaceutical Codex	British Pharmacopoeia	National Formulary
Cortisone	1954	1955	1955
ACTH	1957	1955	1957
Prednisone	1957	1958	1957

Medical progress in England and Wales

Medical progress still continues and no one doubts that a variety of changes has combined to improve the standard of health in England and Wales. Despite this, the annual death rate per 1000 of the population aged 75–85 years has varied very little; it stood at 94·1 in 1948 and 94·7 in 1972. Nor has there been much of a fall in the annual deaths of those aged 55–65 years, the figure per 1000 was 16·8 in 1948 and 15·4 in 1972. The striking drop in deaths from bullous diseases stands out clearly against this background, and also against the slow fall in deaths from non-bullous, non-infective skin diseases over the same period (Fig. 6) (from 1950 to 1967 categories 700 to 716 minus category 704).

One possible reason for this is the introduction of corticosteroids into the treatment of pemphigus and pemphigoid. Adrenal cortex extract had been used with partial success in pemphigus in 1940

(Talbott, Lever & Consolazio, 1940). Cortisone itself (Kendall's Compound E) was isolated in 1941, but waited until 1949 for its first clinical success—in rheumatoid arthritis (Hench *et al.*, 1949). Soon cortisone, other steroids and ACTH were being tested in skin disease. A Medical Research Council panel on the dermatological applications of ACTH and cortisone was set up in 1950, but did not report until 1954 (Medical Research Council, 1954). Meanwhile the first treatment of pemphigus with ACTH and cortisone was reported (Thorn *et al.*, 1950). Some workers took up systemic steroids very quickly; Nelson & Brodey (1955), for example, started in 1949. In England, Church (1960) used corticotrophin for pemphigoid between 1952 and 1954, but changed to cortisone in 1954, and to prednisolone in 1955. Several groups in other countries had used steroids since 1950 (Conrad, Greenhouse & Weiss, 1954; Sulzberger *et al.*, 1951; Reznick, Lever & Frazier, 1956; Combes, 1955) but there must always be a lag between the early trials of a new treatment and its general use. It is not likely that systemic steroids were widely used for bullous diseases in England, apart from centres with special supplies, before they had gained a place in the national publications for pharmacists and doctors. Table 2 shows when cortisone, ACTH, and prednisolone were first mentioned in the *British Pharmaceutical Codex*, the *British Pharmacopoeia* and the *National Formulary*.

The earliest dates of the packaging leaflets issued by one large manufacturing pharmaceutical firm were as follows: cortisone acetate tablets—May 1955; prednisone acetate tablets—May 1956. It is this more widespread use of cortisone which coincides with the sudden halving of deaths from bullous diseases which took place in 1955 in England and Wales. Prednisone and prednisolone took over from cortisone as the mainstay of treatment between 1955 and 1961, but the figures did not improve during this time. The further drop in the early 1960s, maintained until the present, supports the view (Lever, 1972) that higher starting doses of steroids have improved rather than worsened mortality—at least in England and Wales, and in the United States.

Other factors will have been at work too. The major falls in mortality from sepsis in England and Wales took place much earlier: from erysipelas, between 1935 and 1940; and from carbuncles, between 1940 and 1948. These are likely to have been related to the introduction of sulphonamides and penicillin. By 1952, the *National Formulary* already included penicillin, streptomycin, chlortetracycline and chloramphenicol, as well as sulphonamides.

Before systemic steroids were widely used, chlortetracycline was found to be of some help in bullous diseases through an action other than that of simply reducing sepsis (Bettley & Fairburn, 1951). However, the correlation between the changes in mortality from bullous diseases and the introduction of antibiotics is not as good as that with the systemic steroids; nevertheless the improved handling of antibiotics which came with experience may well have played some part. The techniques of regulating fluid and electrolyte balance were already well established by the 1950s and probably contributed little to the sudden fall in mortality in 1955.

DISCUSSION

Figures derived in this way can only be accepted with great caution, and must be viewed in the light of changes in the standard and pattern of medical care, and of medical documentation. This requires a detailed personal knowledge of the country concerned, and, for this reason, discussion here has been mainly confined to the figures from England and Wales.

Hovering over this study is the spectre of inaccurate certification. There is no way, at this stage, of checking back to sift out wrong diagnoses; but a detailed study of the case records of patients listed as dying from bullous diseases in England and Wales between 1962 and 1969 (Savin, unpublished observations) suggests that the main problem is the way in which the words pemphigus and pemphigoid were used interchangeably on the death certificates, rather than the inclusion of cases with some other diagnosis. As both are in the same diagnostic category, this should not affect the issue here. Most patients died in hospital, and were under the care of a dermatologist, so investigation, clinical diagnosis and documentation were usually good.

Mortality figures can reflect changes in treatment: a recent important example was the increased mortality from asthma among young people in England and Wales between 1960 and 1965 (Speizer, Doll & Heat, 1968). The death rate from diabetes fell sharply when insulin was introduced in 1922. The fall in deaths from bullous diseases, at least in England and Wales, and in the United States, seems to be another example, and to be firmly tied to the introduction of systemic steroids. The mortality rate has now fallen to about one-third of the 1950 level. This approach gains value because of the large numbers of patients included, and because it reflects the general experience, and not just that of centres with special experience and skill in the management of these diseases. However, it must suffer because pemphigus and pemphigoid, diseases separate clinically, and needing different steroid schedules, are lumped into the same diagnostic category. It is to be hoped that this position will change when the classification of diseases is next revised.

Pemphigus and pemphigoid are not common: together they made up about 1 in 1700 of skin diseases seen at St John's Hospital for Diseases of the Skin between 1952 and 1965 (Neves, 1966). No one regrets this rarity but it means that most dermatologists can only have dealt with relatively few patients themselves, and must rely heavily on the advice of others who have treated more cases. Yet there is still debate, even between centres with this special experience, about the best line of treatment. Over-vigorous regimes may carry such severe side effects that these outweigh the benefits. Ryan (1971) and Roenigk & Deodhar (1973) felt that their pemphigus results were better with the moderate steroid doses of the 1950s, than with the higher doses used in the 1960s. Lever's view (Lever, 1972) is the opposite. The introduction of anti-mitotic agents (Lever & Goldberg, 1969) also led, in some early trials, to high death rates.

The drop in the overall mortality figures is due to an improved management of both pemphigus and pemphigoid, but the contribution of each cannot be accurately assessed. However, the fall has been most marked in the younger age groups which are likely to contain a high proportion of pemphigus cases, and the continued improvement throughout the 1960s may have been related to the higher steroid dosage schedules being then brought into use.

Pemphigus in the pre-steroid era was usually fatal, but now an analysis of six recent series (Roenigk & Deodhar, 1973) suggests an overall mortality of about 50%, with some workers (Lever, 1972; Krain, 1974) having fewer deaths. Pemphigoid untreated with steroids had a mortality of about 30% (Rook & Waddington, 1953), and this has improved with steroids to as low as 5% in one large series (Sanders & Nelson, 1965). Nevertheless pemphigus and pemphigoid are both still important causes of death, each contributing about the same number of deaths per annum in England and Wales (Savin, unpublished observations). The degree of improvement in the national figures matches fairly closely the falls in mortality seen in individual series from single centres.

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REFERENCES

ARMITAGE, P. (1971) *Statistical Methods in Medical Research*, p. 387. Blackwell Scientific Publications Oxford.

BARLING, B. (1944) Three and a half years treatment with sulphapyridine in a case of dermatitis herpetiformis. *Lancet*, **i**, 503.

BETTLEY, F.R. & FAIRBURN, E.A. (1951) Treatment of some bullous diseases with aureomycin and chloramphenicol. *British Medical Journal*, **ii**, 326.

BRITISH MEDICAL JOURNAL (1971) Swollen figures. *British Medical Journal*, **ii**, 486.

CALNAN, C.D. (1954) A note on sulphone treatment of dermatitis herpetiformis. *Transactions of St John's Hospital Dermatological Society*, **38**, 53.

CHURCH, R. (1960) Pemphigoid treated with corticosteroids. *British Journal of Dermatology*, **72**, 434.

COMBES, F.C. (1955) in discussion of: NELSON, C.T. & BRODEY, M. (1955) Cortisone and corticotropin in treatment of pemphigus. *Archives of Dermatology*, **72**, 495.

CONRAD, A.H., GREENHOUSE, J. & WEISS, R.S. (1954) Treatment of pemphigus with cortisone by mouth. *Archives of Dermatology*, **69**, 66.

HENCH, P.S., KENDALL, E.C., SLOCUMB, C.H. & POLLEY, H.F. (1949) Effect of hormone of adrenal cortex and of pituitary adrenocorticotrophic hormone on rheumatoid arthritis. *Proceedings of the Staff Meetings of the Mayo Clinic*, **24**, 181.

KRAIN, L.S. (1974) Pemphigus. Epidemiologic and survival characteristics of 59 patients 1955–73. *Archives of Dermatology*, **110**, 862.

LEVER, W.F. (1953) Pemphigus. *Medicine, Baltimore*, **32**, 1.

LEVER, W.F. (1972) Methotrexate and prednisone in pemphigus vulgaris. *Archives of Dermatology*, **106**, 491.

LEVER, W.F. & GOLDBERG, H.S. (1969) Treatment of pemphigus vulgaris with methotrexate. *Archives of Dermatology*, **100**, 71.

LIM, C.C., MACDONALD, R.H. & ROOK, A.J. (1968) Pemphigoid eruptions in the elderly. *Transactions of St John's Hospital Dermatological Society*, **54**, 118.

MEDICAL RESEARCH COUNCIL PANEL ON THE DERMATOLOGICAL APPLICATIONS OF A.C.T.H. AND CORTISONE (1954) Treatment of skin disorders with A.C.T.H. and cortisone. *British Medical Journal*, **ii**, 1307.

NELSON, C.T. & BRODEY, M. (1955) Cortisone and corticotropin in treatment of pemphigus. *Archives of Dermatology*, **72**, 495.

NEVES, H. (1966) Incidence of skin diseases 1952–1965. *Transactions of St John's Hospital Dermatological Society*, **52**, 255.

PRAIS, S.J. (1967) A note on the standardized mortality rates for some Jewish communities. *The Jewish Journal of Sociology*, **9**, 66.

PRAIS, S.J. & SCHMOOL, M. (1968) The size and structure of the Anglo-Jewish population 1960–1965. *The Jewish Journal of Sociology*, **10**, 5.

REZNICK, L., LEVER, W.F., & FRAZIER, C.N. (1956) Treatment of pemphigus with A.C.T.H., cortisone and prednisone. *The New England Journal of Medicine*, **255**, 305.

ROENICK, H.J. & DEODHAR, S. (1973) Pemphigus treatment with Azathioprine. *Archives of Dermatology*, **107**, 353.

ROOK, A. & WADDINGTON, E. (1953) Pemphigus and pemphigoid. *British Journal of Dermatology*, **65**, 425.

RYAN, J.G. (1971) Pemphigus—a twenty-year survey of experience with 70 cases. *Archives of Dermatology*, **104**, 14.

SANDERS, S.L. & NELSON, C.T. (1965) Pemphigus and pemphigoid. *Medical Clinics of North America*, **49**, 681.

SPEIZER, F.E., DOLL, R. & HEAF, P. (1968) Observations on recent increase in mortality from asthma. *British Medical Journal*, **i**, 335.

SULZBERGER, M.B., SAVER, G.C., HERRMANN, F., BAER, R.L. & MILBERG, I.L. (1951) Effects of A.C.T.H. and cortisone on certain diseases and physiologic functions of the skin. *Journal of Investigative Dermatology*, **16**, 323.

TALBOTT, J.H., LEVER, W.F. & CONSOLAZIO, W.V. (1940) Metabolic studies on patients with pemphigus. *Journal of Investigative Dermatology*, **3**, 31.

THORN, G.W., FORSHAM, P.H., FRAWLEY, T.F., HILL, S.R., ROCHE, M., STAEHELIN, D. & WILSON, D.L. (1950) The clinical usefulness of A.C.T.H. and cortisone. *New England Journal of Medicine*, **242**, 783, 824 and 865.

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Pharmacology and Treatment

A new model for human bioassay of topical corticosteroids

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SUMMARY

The ability of topical corticosteroids to reduce the size of weals induced with histamine on human skin was used as a human bioassay model to assess their relative efficacy. Histamine was introduced by the pinprick method and various topical corticosteroids were assessed. It is a simple, easy and reliable procedure which can be used on both white and dark people. Even though it is a non-clinical assay, it possesses close similarity to processes involved in the development of clinical dermatoses.

Since the systemic and topical potency of corticosteroids do not follow the same pattern, it becomes a problem for a dermatologist to select the proper steroid from the available long list. A practical method to assay topical corticoids which parallels the clinical response is essential. A study was undertaken to evaluate a new, easy and practical human bioassay procedure to establish the comparative efficacy of various topical corticosteroids.

MATERIALS AND METHODS

Young human volunteers aged between 15 and 30 years, having no systemic disease or skin infection, were the subjects of the study. None had used either systemic or topical corticosteroids or antihistamines for at least 8 weeks before the study.

It was possible to produce graded sizes of weals on the skin when it was pricked to a standard depth with a needle through a histamine solution. Prior treatment with topical corticosteroids reduces the size of weal so induced and this was utilized to quantify their comparative efficacy.

The following concentrations of different topical corticosteroids were selected (therapeutic concentrations) arbitrarily and included in the study:

(1) Hydrocortisone, 1%; (2) Prednisolone, 1%; (3) Betamethasone 17-valerate, 0·1%; (4) Dexamethasone, 0·1%; (5) Beclomethasone dipropionate, 0·1%; (6) Flumethasone pivalate, 0·1%; (7) Triamcinolone acetonide, 0·1%; (8) Flurandrenolide, 0·1%.

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The pure steroids under study were dissolved in alcohol in the above concentrations. The solutions were transferred to amber-coloured vials, coded, capped with rubber lids and refrigerated. Five sites were chosen on each forearm. 0·25 ml of each of the coded steroid solutions was poured with separate tuberculin syringes onto the different sites where circular glass cups were placed. One site on each forearm was kept as control and was treated with the solvent (absolute alcohol) only. All applications of the steroid solutions were done in a random fashion to exclude site to site variation and in a double blind manner to avoid personal bias. The sites were marked and occluded with separate polythene patches by applying pieces of cotton-backed adhesive tape at the borders. The whole forearm was wrapped with an elastic-crêpe bandage to keep the occlusion in proper position. After 18 h the occlusion was removed and the sites were cleaned with soap and water. After the erythema due to sticking plaster had subsided, i.e. after roughly 1 h, the assay was performed as follows:

A circular glass cup with open ends was placed over the control site. 0·5 ml of 0·1% histamine acid-phosphate solution was transferred to the centre of the cup and a prick was made with an automatic pricking needle. The solution was allowed to stay at the site for 30 s and then the site was cleaned gently with cotton wool. The length of the needle was kept constant at 2 mm throughout the study. Tracing of the weal was made on transparent paper 6 min after the prick. The average diameter of the weal was recorded after measuring the minimum and maximum diameters. The weals were roughly circular. The surface area of the weal was then calculated. The steroid treated sites were similarly tested and the surface areas of their weals calculated.

RESULTS AND DISCUSSION

Table 1 summarizes the histamine response of the skin, represented as surface area of the weal in mm^2, before and after application of alcoholic solutions of various topical corticosteroids with mean, s.d., F and P value, critical difference, percentage of potency and potency ratio.

The present study revealed that there was a statistically significant difference between the histamine suppressor activity of various steroids and the control ($P<0{\cdot}001$). For the purpose of comparison,

TABLE 1. Showing the surface area of weals, i.e. response to histamine pin-prick, before and after application of various topical steroids

Steroid	No. of sites tested	Histamine response of skin (range)	Mean	s.d.	% of potency	Potency ratio
Control	20	7·07–19·64	12·79	3·62	—	—
Hydrocortisone 1%	20	4·91–19·64	10·07	3·29	20·3	1·0
Prednisolone 1%	20	4·91–12·57	8·15	2·27	30·63	1·7
Betamethasone 17-valerate 0·1%	20	3·14–12·57	6·14	2·96	52·0	24·0
Dexamethasone 0·1%	20	3·14–12·57	5·97	2·43	53·3	25·0
Beclomethasone dipropionate 0·1%	20	3·14–11·31	6·32	2·35	50·6	23·0
Flumethasone pivalate 0·1%	20	3·14–9·62	6·32	1·67	50·6	23·0
Triamcinolone acetonide 0·1%	20	2·27–12·57	6·99	2·29	45·3	21·0
Flurandrenolide 0·1%	20	2·51–12·57	6·29	2·29	50·82	23·8

F = 6·29; $P<0{\cdot}001$; C. at 0·05% = 1·704.

hydrocortisone was assigned a potency of 1 and the rest of the steroids were compared to it. The order of topical efficacy in terms of histamine suppression increases from hydrocortisone as 1, prednisolone 1·7, triamcinolone acetonide 21, flumethasone pivalate 23, beclomethasone dipropionate 23, flurandrenolide 23·8, betamethasone 17-valerate 24, dexamethasone 25.

The histamine bioassay of topical corticosteroids has many advantages over the other known assay techniques. It is a simple, reliable, and reproducible procedure. More than two or three compounds may be tested at a time on a single subject and hence the study may be conducted even with a smaller number of test subjects. The end point of the assay is very easy to observe. Skin colour does not interfere with the interpretation of the results. Even though it is a non-clinical human assay, it resembles clinical conditions closely having some similarity to the processes involved in the development of inflammatory dermatoses.

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The inhibiting effect of soft paraffin on the Köbner response in psoriasis

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SUMMARY

White soft paraffin has been shown to inhibit the development of the isomorphic response (Köbner phenomenon) in psoriasis. The possible reasons for this are discussed and it is suggested that this finding has implications which should lead to a greater understanding of the nature of psoriasis.

Despite intense efforts throughout the world the causation of psoriasis remains a baffling problem (Shuster, 1971). Two main facts seem fundamental—that genetic factors play an important role (Watson, Cann & Farber, 1972), and that certain trigger factors may provoke the psoriatic reaction in the individual so constituted. Trigger factors may be systemic, e.g. streptococcal infections (Whyte & Baughman, 1964) or local, such as physical injury. The latter is often called the Köbner or isomorphic response, and is clinically and histologically indistinguishable from spontaneous lesions, apart from its shape, which closely follows the area traumatized. Classically it follows 6–18 days after injury, which may be a scratch, sunburn, abrasion or operation wound. It has been shown that both epidermis and dermis must be damaged before the reaction occurs (Stankler, 1969). Since there is no animal model of psoriasis, the Köbner effect provides the only controllable method currently available for research into this common disorder. Consequently it has been extensively investigated with regard to the type, degree and depth of injury producing it, the time course between stimulus and response and the appearances at the clinical, histological and electron microscopical level. Many chemicals and drugs have been used in attempts to suppress the reaction, without success. It is obvious that if a method were available to prevent or suppress the reaction, it would be of the greatest possible significance, both theoretically and at the clinical level where it might well prove a source of practical techniques in the management of the spontaneous disease.

A positive response indicates active disease, and multiple simultaneous stimuli anywhere on the skin produce an equal number of responses (Pedace, Muller & Winkelmann, 1969). This observation is of great significance and accords well with observations suggesting that psoriasis is latent throughout the whole integument even when only localized plaques are visible clinically.

The following experiments were designed to test the hypothesis that a positive Köbner reaction is due to local overgrowth of bacterial organisms (especially streptococci) at the site of trauma. If this were so it would correlate well with the guttate psoriasis reactions seen after systemic strepotococcal infections, the individual lesions here being produced possibly by streptococcal products released into the blood stream and settling in the skin. As will be seen, the results do not support this hypothesis at all, but instead show that soft paraffin used as an ointment base has a significant inhibiting effect on the Köbner response.

MATERIALS AND METHODS

Subjects

These were 104 adult patients with active psoriasis who had given their informed consent to the procedures used. Patients receiving antibiotics, sulphonamides or antimitotics (e.g. methotrexate or hydroxyurea) were not included. Routine topical therapy was given, avoiding the test areas of skin. Neobacrin ointment (Glaxo) containing 5 mg of neomycin and 500 units bacitracin zinc in each gram of paraffin base was supplied in tubes identical to those containing only the base. These preparations were coded and the key kept by the pharmacy staff. The stimulus consisted of a short (1 cm approx.) single scratch or cross made with a sterile needle on the upper outer arm or back. The response was read at 7, 14 and 21 days usually, but this varied according to the individual patient's convenience. The greatest response in each scratch was then read as negative, doubtful (?) or positive (+, ++, or +++) and these were scored as 0, 0·5, 1, 2, 3 respectively.

Five sets of experiments were done:

(1) Nine subjects were tested on one site only and the scratch lesion was cultured for bacterial organisms on the 1st, 3rd and 7th day after stimulus (day 0).

(2) Sixteen patients were tested on both upper outer arms and one of these sites after scratching was treated with Neobacrin ointment twice daily for 3 weeks, the other side being left untreated. The results were read blind, that is, without the observer being aware which side had been treated.

(3) Eighteen patients were tested as in (2) but one side was treated with Neobacrin ointment and the other with its base again twice daily for 3 weeks, using a double-blind system.

(4) Thirty-one patients were tested as in (3) but with an additional stimulus over the back, which was left untreated. The results were read 'blind' as in (2) above.

(5) Thirty patients were tested as in (2) but the base was used instead of Neobacrin ointment, the other side being left untreated. The results were read 'blind' as in (2).

RESULTS

The data have been analysed using 'the sign test' for non-parametric observations.

Experiment 1

None of the lesions gave positive cultures for pathogenic organisms. Eight of the nine patients gave a positive Köbner reaction.

Experiment 2 (*Table* 1)

Seven of the sixteen patients gave positive reactions, all on the untreated side. These results show a significant inhibitory effect of Neobacrin compared with the untreated sites ($P = 0{\cdot}006$ using a two-tailed test).

Experiment 3 (*Table* 2)

Four of the eighteen patients tested gave positive reactions as follows:

Control side (ointment base), 4; Neobacrin side, 3.

There is no significant difference between Neobacrin ointment and its base in these experiments.

TABLE 1. Effect of Neobacrin ointment on the Köbner response in sixteen patients

Untreated site	Neobacrin treated site
+	–
+	?
+++	?
+	–
+	–
?	–
–	–
?	–
+	–
+	–
–	?
–	–
–	–
?	–
–	–
?	–

Scoring system: negative – = 0; doubtful (?) = 0·5; positive + = 1·0; ++ = 2·0; +++ = 3·0.

Results: two-tailed sign test gives $p = 0{\cdot}006$ indicating Neobacrin has a significant effect on the Köbner response.

TABLE 2. Comparison of Neobacrin and its base on the Köbner response in eighteen patients

Site treated with base	Site treated with Neobacrin
Negative (14)	Negative (14)
+	+
+	–
+	+
++	+

Comment: no significant difference between Neobacrin and its base.

Experiment 4 (*Table* 3)

These results confirm that there is no significant difference between the effect of Neobacrin and that of its base on the Köbner response, and that Neobacrin inhibits the response compared with untreated sites. Additionally it is shown that the ointment base itself has a significantly inhibitory effect on the Köbner reaction. Table 4 shows that this effect is of approximately the same degree as that of the Neobacrin ointment.

Experiment 5 (*Table* 4)

These results confirm the previous findings of Experiment 3 (Table 4) that the base inhibits the Köbner response.

DISCUSSION

This series of experiments gave unexpected results but it is possible that these are more significant than those looked for would have been. It is clear that the ointment base—soft paraffin—has a substantial inhibiting effect on the Köbner response, which in many cases prevented the reaction completely. We have considered a number of possible explanations.

1. *Epidermal hydration.* It is known that soft paraffin increases the hydration of epidermis and that this is also one of the chief effects of polythene occlusion (Anderson *et al.*, 1973). The latter tends to decrease mitotic rate and produce partial resolution of the psoriatic plaques. Since our own work was begun it has been reported that simple ointment bases may also decrease mitotic rate and labelling index in stimulated mouse epidermis (Tree & Marks, 1974). The common mechanism of epidermal hydration may be significant in all of these and our own observations.

TABLE 3. Comparison of effect on Köbner response in thirty-one patients of (a) Neobacrin (b) base (c) no treatment

Site treated with Neobacrin	Site treated with Base	Untreated site
Negative (14)	Negative (14)	Negative (14)
−	−	+
−	+	+
?	?	+
?	+	?
−	−	+
+	+	+
+	+	+
+	+	+
?	?	+
+	+	+
−	−	++
+	+	+
+	−	?
+	+	+
?	+	+
?	−	+
+	+	+

Scoring system: see Table 1.
Results: (1) Since Neobacrin previously shown to inhibit Köbner response, can use one-tailed test which gives $P = 0{\cdot}02$ indicating Neobacrin significantly inhibited Köbner response, confirming previous experiment. (2) There is no significant difference between Neobacrin and its base, confirming previous experiment. (3) Using a one-tailed test, base significantly inhibited Köbner response, $P = 0{\cdot}035$.

2. *Bacterial hindrance.* Inunction of a greasy ointment base may physically entrap bacteria which would otherwise invade and multiply in damaged tissue. This supposes that bacteria do play a role in the Köbner response; one would have expected in this case some discernible difference between the inert base and base plus a powerful combination of antibiotics, which is certainly not apparent in the present data.

3. *Oxygen lack.* An impermeable layer of paraffin might prevent ready access of molecular oxygen to a rapidly metabolizing tissue (Hammer & Hellerström, 1968). This implies a requirement for and ability to utilize atmospheric molecular oxygen by damaged epidermis.

4. *Retention of mitosis inhibitors.* The sealing effect of paraffin might reduce or prevent loss of chalone or some such mitosis inhibitor. Its absorption back into the dividing zone of the epidermis could be enhanced by increased hydration as in (1) above.

5. *Specific chemical effect.* There could be a direct chemical effect of the soft paraffin—or some component—on the psoriatic process.

We believe that whatever the ultimate explanation, this observation has far-ranging implications which might provide a springboard for further advances in the field of psoriatic research.

TABLE 4. Comparison of Köbner response in untreated sites and sites treated with base only in thirty patients

Untreated control site	Treated site (base only)
+++	+
—	—
?	—
—	—
—	—
—	—
?	?
—	—
?	—
?	—
—	—
+	—
—	—
+	+
++	+
?	+
—	?
+	?
+	+
?	—
—	—
+	—
?	++
?	—
?	—
—	—
—	—
+	+
—	—
?	—

Scoring System: see Table 1.
Results: One-tailed test gives $P = 0{\cdot}018$ indicating that the base significantly inhibited Köbner response.

TABLE 5. Overall scores for Köbner responses for all sites tested

Neobacrin	—	19 out of 65 tested	=	29·2%
Base	—	25·5 out of 79 tested	=	32·3%
Untreated	—	44 out of 77 tested	=	57·1%

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REFERENCES

ANDERSON, R.L., CASSIDY, J.M., HANSEN, J.R. & YELLIN, W. (1973) The effect of *in vivo* occlusion on human stratum corneum hydration-dehydration *in vitro*. *Journal of Investigative Dermatology*, **61**, 375.

HAMMER, H. & HELLERSTRÖM, C. (1968) Oxygen consumption of the germinal epithelium in psoriatic human skin as measured by the Cartesian diver micro-gasometer. *Acta dermato-venereologica*, **48**, 563.

PEDACE, F.J., MULLER, S.A. & WINKELMANN, R.K. (1969) Biology of psoriasis: experimental study of the Köbner phenomenon. *Acta dermato-venereologica*, **49**, 390.

SHUSTER, S. (1971) Research into psoriasis—the last decade. *British Medical Journal*, **iii**, 236.

STANKLER, L. (1969) An experimental investigation on the site of skin damage inducing the Köbner reaction in psoriasis. *British Journal of Dermatology*, **81**, 534.

TREE, S. & MARKS, R. (1974) An explanation for the 'placebo' effect of bland ointment bases. *British Journal of Dermatology*, **92**, 195.

WATSON, W., CANN, H.M. & FARBER, E.M. (1972) The genetics of psoriasis. *Archives of Dermatology*, **105**, 197.

WHYTE, H.J. & BAUGHMAN, R.D. (1964) Acute guttate psoriasis and streptococcal infection. *Archives of Dermatology*, **89**, 350.

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Case Reports

Cutaneous alternariosis

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SUMMARY

Two patients with cutaneous alternaria infection are presented. In both patients the skin lesions were characterized by multiple non-healing ulcers covered with dry crusts. Although the skin changes were macroscopically alike in the two patients, differences in the histology were seen. Both patients had primary debilitating diseases. A review of the literature is presented and revealed an additional ten cases of cutaneous alternariosis. Methods for the isolation of *Alternaria* and the susceptibility of the fungus to antimycotic drugs are presented.

During recent years there has been an increasing number of reports concerning opportunistic fungal infections. These infections may affect the skin (Bach *et al.*, 1973; Haim *et al.*, 1973) as well as visceral organs (Field, 1968; Bach *et al.*, 1973). Thus fungi normally considered as saprophytes or commensals in man have been found able to establish infections, particularly in the 'compromised host'. Such fungi have included *Aspergillus*, *Candida*, *Nocardia* (Brönnestam & Hallberg, 1965; Field, 1968; Bach *et al.*, 1973; Haim *et al.*, 1973) and *Mucor* (Haim *et al.*, 1973).

Alternaria is a well-known plant pathogen (Neergaard, 1945; Park, 1968) which may cause allergic manifestations in man, such as bronchial asthma (Gravesen, 1972) and hypersensitivity pneumonitis (Schlueter, Fink & Hensley, 1972). Yu (1965) found *Alternaria* to occur on normal skin. Botticher (1966) isolated *Alternaria* from several cases with superficial mycotic skin lesions, but only in a few of the patients was *Alternaria* considered to be the causative organism.

In the present report skin infection with *Alternaria* is described in two patients with a primary debilitating disease. A search of the literature is presented and revealed an additional ten cases of cutaneous alternariosis (Table 1).

PATIENTS

Case 1

A 23-year-old female (A.W.), preparatory school teacher, who earlier had been in a good state of health. At the age of 21 years she experienced attacks of dizziness or syncope in connection with

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TABLE 1. Cases of cutaneous infections with *Alternaria* reported in the literature

Author(s)	Year of publication	Patient's age	Sex	Location of lesions	Histological examination
Borsock	1933	36	F	Forearm and hand	ND
Takatsuki	1938	15	F	Foot, leg, forearm and face	+
Botticher	1966	2	M	Cheek	ND
		2	F	Scalp	ND
Delacrétaz *et al.*	1970	35	M	Nails	ND
		3	M	Nails	ND
		24	M	Hands	ND
Kawasaki *et al.*	1970	13	F	Cheek	+
Nakama	1970	56	F	Nose	+
Higashi & Asada	1973	1/2	F	Cheeks	+

ND = not done.

physical exercise. Oedema of the feet occurred 6 months later, and the patient was referred to the Department of Cardiology, Lund. The examination revealed a pulmonary hypertension of unknown aetiology. No infiltrates of the lungs were ever found on chest X-ray examinations. The disease continuously progressed and she died from cardiac insufficiency. Autopsy was prohibited by the relatives.

Four and a half months before the patient's death an abscess developed on the dorsum of her oedematous left foot. *Staphylococcus aureus* was cultivated from the lesion and she was treated with large doses of penicillin, but the ulcer persisted. One month later the skin of the knuckles of both hands and knees became erythematous and eroded with formation of crusted ulcers. She was first seen

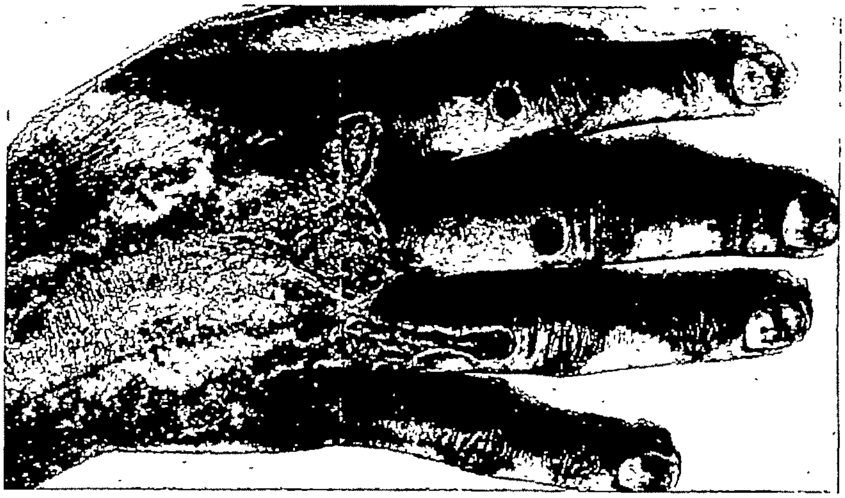

FIGURE 1. Cutaneous alternariosis. Case 1. Persisting ulcers after removal of crusts.

at the Department of Dermatology 2½ months before her death. Under the dry, firmly fixed crusts, shallow moderately weeping ulcers were found (Fig. 1). On the dorsum of the left foot there was a large open ulcer with a base of granulation tissue.

Case 2

A 63-year-old mill worker (I.P.), who had proteinuria as a child. He was examined 9 years ago in a health survey and proteinuria as well as a slightly reduced renal function were then detected. Further examination including renal biopsy demonstrated a proliferative glomerulonephritis. His condition changed for the worse and 4½ years later haemodialysis twice a week was started. During the subsequent 3 years his condition further deteriorated. At the age of 61 years he received a renal transplant and was then given 25–50 mg of azathioprine and 10–40 mg of prednisone daily. He died with a pancytopenia 1½ years after the transplantation.

Eleven months before he died, non-healing ulcers developed on the backs of his hands and fingers and on his elbows. After a further 2 months he was seen at the Department of Dermatology, Lund. In the regions mentioned, he had ulcers covered with crusts (Fig. 2), a few haemorrhagic pustules and yellow papules.

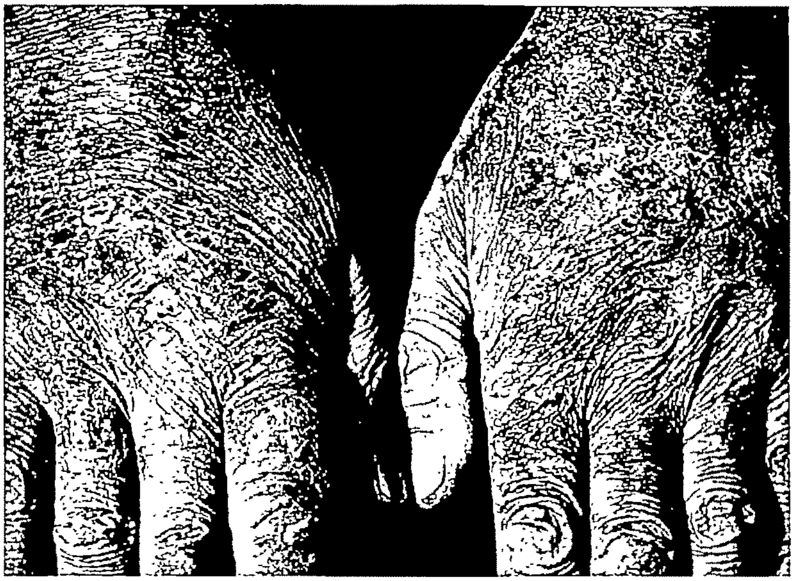

FIGURE 2. Cutaneous alternariosis. Case 2. Untreated, crusted ulcers of the hands.

METHODS

Sampling and fungus isolation procedures

Sampling for fungal cultures from both patients was made from several lesions on various occasions by scrapings from the ulcers. Cultures were also made from a skin biopsy in Case 1. In some instances, sampling was made with sterile cotton-tipped swabs and inoculated 'bedside' onto solid culture media.

The clinical specimens were inoculated onto the following media: Sabouraud agar (Oxoid), Sabouraud agar containing 0·5 μg/ml of cycloheximide, blood agar plates supplemented with 50

IU/ml of penicillin and 50 μg/ml of streptomycin, and Mycological agar (Difco). Incubations were regularly made at 30°C, but in some instances also at 20 and 37°C. Isolated strains were subcultured onto the above media as well as on Sabouraud agar containing a pH-indicator (bromcresol purple). Bacterial cultures, including cultures for mycobacteria, were also made from specimens collected from the skin lesions.

Antimycotic susceptibility tests

The susceptibility of isolated *Alternaria* strains to amphotericin B (Squibb), griseofulvin (Lövens kemiska fabrik, Ballerup, Denmark), 5-fluorocytosine (Hoffmann-LaRoche) and nystatin (Squibb) was determined. The substances were added in two-fold increasing concentrations to Sabouraud agar medium. The susceptibility to 5-fluorocytosine was, however, tested on yeast morphology agar (Difco) by the method recommended by the manufacturer of the drug. The test was read after 2–3 days incubation at 37°C and the minimum inhibitory concentration was considered to be the lowest concentration of the drug causing complete inhibition of growth.

Animal pathogenicity tests

In both patients material collected from the ulcers was inoculated into the hind leg of guinea-pigs. The guinea-pigs were sacrificed after 6 weeks.

One strain of *Alternaria*, isolated from Case 2, was injected intracutaneously, intraperitoneally and intrathecally into mice.

Test for precipitating antibodies to Alternaria

Serum samples from Case 2 were tested for precipitating antibodies to a commercial antigen of *Alternaria* (Miles Lab., Ltd.) using Ouchterlony's double diffusion technique in agarose gel.

Histopathological examination

In Case 1, punch biopsy samples were taken from an ulcer on the hand and from a blister on the foot. In Case 2, a punch biopsy was taken from a papule on the elbow. Multiple sections from each biopsy were stained with haematoxylin–eosin, Alcian blue–Kernechtrot, PAS (McManus), with methenamine–silver nitrate according to Gomori, and according to van Gieson. Autopsy was performed in Case 2.

RESULTS

Mycological studies

In both patients abundant growth of *Alternaria* was obtained from all skin lesions examined. On one occasion, *Candida guillermondi* and *Candida parapsilosis* were also found in one of the skin lesions of Case 1. In Case 2, no other fungi were ever isolated. As already mentioned, *S. aureus* was isolated from an abscess on the foot of Case 1.

Isolated strains of *Alternaria* grew on all media used except for the medium containing cycloheximide. The strains grew on plates incubated at 20°C as well as at 30°C, while only scanty growth was obtained on the plates incubated at 37°C.

In Case 1, the isolated strains belonged to the species of *Alternaria alternata* (Fr. Keissler) (Fig. 3). (The diagnosis was confirmed by 'Centralbureau voor Schimmel-cultures', Baarn, The Netherlands.) In Case 2, the fungi isolated belonged to the genus of *Alternaria*, but unfortunately the strains from this patient succumbed before they could be identified as to species.

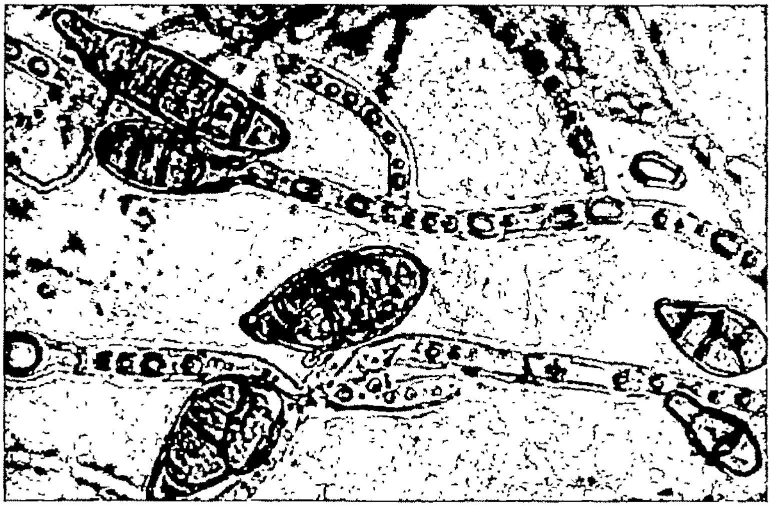

FIGURE 3. Microscopic appearance of *Alternaria alternata* grown on Sabouraud agar at 30°C for 10 days. Hyphae and conidia with transverse and longitudinal septae. The length of the conidia is 10–20 μm.

Susceptibility of isolated strains to antifungal drugs

The minimum inhibitory concentration (MIC) of some antifungal drugs was investigated for three strains of *Alternaria* from patient 1 (A.W.) and two strains from patient 2 (I.P.). For each drug the strains from both patients showed about equal MIC values (amphotericin B 1·6–3·2 μg/ml, griseofulvin 10 μg/ml or more, and nystatin 8–32 μg/ml). Strains from patient 2 were resistant to 10 μg/ml of 5-fluorocytosine, strains from patient 1 not being tested against this drug.

Test for precipitating antibodies to Alternaria

The serum samples of Case 2 contained no precipitating antibodies demonstrable with the alternaria antigen used.

Animal pathogenicity tests

The mouse pathogenicity tests of a strain of *Alternaria* isolated from Case 2 did not reveal any signs of infection in the injected mice.

No signs of mycobacterial infection were found in guinea-pigs inoculated with samples collected from ulcers of the two patients.

Treatment

Patient A.W. (Case 1) was initially treated with dressings soaked with potassium permanganate and ointment containing sodium fusidate (Fucidina, Lövens). When the fungal diagnosis had been established local treatment with powder containing chlorquinaldol (Sterosan, Geigy) was started without success.

The other patient (I.P.) was initially given a similar treatment. It was later changed to gentian violet solution and nystatin ointment (Mycostatin, Squibb), but no improvement was observed.

Histopathology

Case 1. In the biopsy from the back of the hand, the stratum corneum and especially the upper and

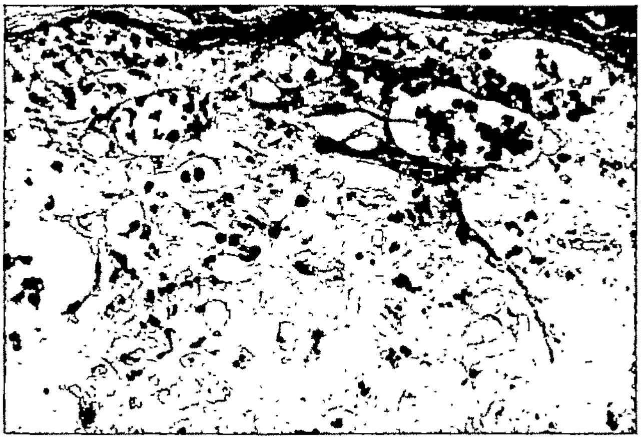

FIGURE 4. Cutaneous alternariosis. Skin biopsy from the back of the hand of Case 1, showing several intra-epidermal abscesses and fungal hyphae reaching down into the stratum spinosum. Haematoxylin–PAS (McManus) (×400).

middle parts of the epidermis showed numerous small abscesses, containing neutrophil granulocytes and some lymphocytes. Septate fungal hyphae (3–5 μm) were seen not only in the stratum corneum but also reaching down into the stratum spinosum (Fig. 4). The dermis showed discrete inflammatory changes with capillary dilatation and perivascular infiltration of granulocytes and lymphocytes. No spores were found.

In the biopsy from the dorsum of the foot a subepidermal blister contained numerous erythrocytes

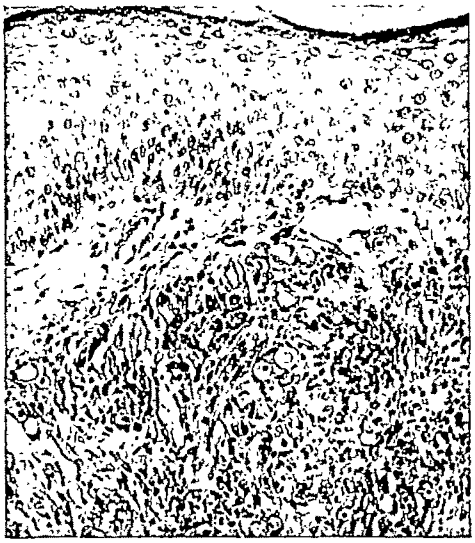

FIGURE 5. Cutaneous alternariosis. Skin biopsy from the elbow of Case 2, showing a granulomatous inflammation in the upper dermis with several fungal spores in the granulation tissue. Haematoxylin–PAS (McManus) (×160).

and a proteinaceous fluid. The dermis showed discrete non-specific inflammatory changes. No fungi could be demonstrated.

Case 2. In the biopsy from the elbow, the upper and middle parts of the dermis were the site of a heavy granulomatous inflammation. The granulation tissue was dominated by histiocytes and also contained some multinucleated cells, fibroblasts and lymphocytes. In the central parts small abscesses were formed. The granulation tissue contained numerous round or oval spore-like bodies (10–15 μm) (Fig. 5), which occasionally showed protrusions suggesting the budding of yeasts. Most of them seemed to be located extracellularly and were surrounded by a rim of histiocytes. In PAS-stained sections their walls were intensely purple and their interior often had a finely granulated structure. Their walls were also intensely blue-stained with Alcian blue and heavily impregnated with Gomori's technique. Also a few hyphae of similar appearance as in Case 1 were detected. Numerous granulocytes invaded the epidermis which showed a reactive hyperplasia.

Autopsy findings in case 2

The autopsy revealed generalized arteriosclerosis, myocardial fibrosis, left ventricular hypertrophy, small contracted end-stage kidneys probably on a glomerulonephritic basis. The transplanted kidney showed nephrocalcinosis, interstitial oedema and fibrosis with foci of lymphoid cells. The glomeruli were hypercellular with thickened capillary walls, and some glomeruli were hyalinized. The bone-marrow was hypocellular with haemorrhages, and there were signs of a generalized haemorrhagic diathesis. No signs of visceral mycosis were seen.

DISCUSSION

In the two cases described the skin lesions were caused by a fungus not commonly regarded as pathogenic in man—*Alternaria*. Searching the literature we found, however, reports of skin infections by *Alternaria* in an additional ten cases (Table 1).

Alternaria is ubiquitous and is frequently found in the environment (Bulmer, 1969; Park, 1968). Gravesen (1972) studied the occurrence of airborne fungi in the homes of Danish children suffering from inhalation allergy and hypersensitivity to fungi. *Alternaria* spp. were found in the majority of homes studied, frequently in the summer months.

Yu (1965) found *Alternaria* to be number eight in frequency of fungi isolated from normal human skin, i.e. in 97 of 3584 isolates. Botticher (1966) found that *Alternaria* spp. made up 15% of all fungi isolated from specimens collected from assumed superficial mycotic lesions. However, only in a few instances was *Alternaria* the only fungus isolated. Two of these patients had skin lesions of appearance similar to those found in our patients. As *Alternaria* frequently occurs in the environment and on normal human skin it could be difficult to establish whether it is a contaminant or not when found in cultures. In the present cases we consider that *Alternaria* was associated with the development of the patients' skin lesions. The fungus could be isolated from several sites on repeated occasions and it was found on cultures from biopsy material, the histological examination of which also revealed a fungus infection.

In the present two cases *Alternaria* was isolated by conventional techniques used for the diagnosis of dermatophytes. Cultures for *Alternaria* have to be performed on media that do not contain cycloheximide or other substances used to suppress the growth of saprophytes; otherwise fungi of this genus may escape detection.

Earlier reported patients with *Alternaria* skin infections (Table 1) have generally been in good health, while our two patients had debilitating diseases. The fungal infections in our patients may be regarded as opportunistic ones, possibly appearing as a consequence of the treatment given. One of the patients (Case 2) had a kidney transplant and was being treated with immunosuppressive drugs, when the mycotic infection appeared. This patient may certainly be regarded as a 'compromised host'. Haim *et al.* (1973) and Bach *et al.* (1973) found skin infections to be common in immunosuppressed patients with kidney transplants, while Noble, Rebel & Smith (1974) examined dialysis and transplant patients but did not find fungal infections to be more common in such patients than in controls.

In Case 1, although not studied in great detail, there was no evidence of impaired immunity. Thus this patient had normal white blood cell counts, normal bone-marrow aspirates and the tuberculin test was positive. She had been treated with large doses of penicillin shortly before the appearance of the multiple ulcers. It is known that opportunistic mycotic infections may be seen in connection with antibiotic treatment (Field, 1968).

Alternaria spp. may contain mycotoxins (Krogh, 1970), which might be of pathogenic significance in skin infections in man. However, we did not test whether the strains isolated from our patients were toxin-producing.

The present patients both had skin lesions located on the backs of the hands. In addition Case 1 had lesions on the knees, and Case 2 on the dorsal aspect of the fingers and the elbows. Two other patients with lesions on the dorsal aspect of the hands have been reported earlier (Borsock, 1933; Delacrétaz, Grigoriu & Grigoriu, 1970, see Table 1). It could also be mentioned that Delacrétaz *et al.* (1970) found two cases of alternaria infections located to the finger nails, but with no skin lesions. In *in vitro* experiments with *Alternaria*, vegetative as well as sporing growth has been demonstrated on keratin and hair (Botticher, 1966). *Alternaria* has also been recovered from the face in five of the ten patients earlier described (Takatsuki, 1938; Botticher, 1966; Kawasaki, Akagi & Nishimura, 1970; Nakama, 1970; Higashi & Asada, 1973).

The appearance of the skin lesion in the present cases, i.e. non-healing ulcers covered particularly by dry, firmly fixed crusts, was similar to those reported earlier (Delacrétaz *et al.*, 1970; Botticher, 1966). Kawasaki *et al.* (1970) and Higashi & Asada (1973) described crusted ulcers and papules of the same type as found in Case 2. Takatsuki (1938) found crusted papules, pustules as well as eroded lesions, while Borsock (1933) and Nakama (1970) observed only papules.

The histological findings in the two cases differed. Case 1 had a picture of a superficial mycosis with a discrete inflammatory reaction in the corium and hyphae were present not only in stratum corneum but also in the deeper layers of the epidermis. Higashi & Asada (1973) reported a similar histological picture. In contrast, the histology in Case 2 showed formation of granulation tissue throughout the corium. The histological picture had some similarity to cutaneous cryptococcosis. Kawasaki *et al.* (1970) described a similar microscopic picture in their case of cutaneous alternariosis. It thus seems obvious that *Alternaria* may have varying morphology not only on culture media but also in tissues.

Takatsuki (1938) and Kawasaki *et al.* (1970) reported on the natural course of skin infection caused by *Alternaria*. The course was characterized by alternating healing and formation of new lesions. Botticher (1966) treated one patient successfully with Wescodyne® (a compound containing iodine) and another with griseofulvin and Whitfield's ointment. Healing was complete within 3 weeks. Delacrétaz *et al.* (1970) cured one patient with topical treatment with phenyl mercury borate and Higashi & Asada (1973) another patient with Pimabucin ointment. The present patients were treated topically with potassium permanganate, gentian violet, chlorquinaldol and nystatin. The crusts fell off during the therapy, but the ulcers did not show any tendency to heal.

In the susceptibility tests with antifungal drugs which might have been used for systemic therapy, i.e.

griseofulvin, amphotericin B and 5-fluorocytosine, low MIC values were found for amphotericin B only. Because of the condition of the patients this drug was, however, not instituted.

ACKNOWLEDGMENT

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REFERENCES

Bach, M.C., Adler, J.L., Breman, J., P'eng, F-K., Sahyoun, A., Schlesinger, R.M., Madras, P. & Monaco, A.P. (1973) Influence of rejection therapy on fungal and nocardial infections in renal transplant recipients. *Lancet*, **i**, 180.

Borsock, M.E. (1933) Skin infection due to *Alternaria tenuis*. *The Canadian Medical Association Journal (New series)*, **39**, 479.

Botticher, W.W. (1966) *Alternaria* as a possible human pathogen. *Sabouraudia*, **4**, 256.

Brönnestam, R. & Hallberg, T. (1965) Precipitins against an antigen extract of *Aspergillus fumigatus* in patients with aspergillosis or other pulmonary diseases. *Acta Medica Scandinavica*, **177**, 385.

Bulmer, G.S. (1969) Fungi and fungus allergy. In: *Clinical Immunology and Allergy* (Ed. by L.H. Criep), 2nd edn, p. 369. Grune & Stratton, New York, London .

Delacrétaz, J., Grigoriu, D. & Grigoriu, A. (1970) *Alternaria tenuis* en pathologie cutanée humaine. *Annales de Dermatologie et de Syphiligraphie (Paris)*, **97**, 15.

Field, M.H. (1968) Opportunistic fungal infections. *Journal of the American Medical Women's Association*, **23**, 529.

Gravesen, S. (1972) Identification and quantitation of indoor airborne micro-fungi during 12 months from 44 Danish homes. *Acta Allergologica*, **27**, 337.

Haim, S., Friedman-Birnbaum, R., Better, O.S. & Tuma, S. (1973) Skin complications in immunosuppressed patients: follow-up of kidney recipients. *British Journal of Dermatology*, **89**, 169.

Higashi, N. & Asada, Y. (1973) Cutaneous alternariosis with mixed infection of *Candida albicans*. *Archives of Dermatology*, **108**, 558.

Kawasaki, H., Akagi, M. & Nishimura, N. (1970) A case of cutaneous alternariosis. *Japanese Journal of Medical Mycology*, **11**, 218.

Krogh, P. (1970) Foderstoffers kontamination med mykotoksiner. *Medlemsblad for den danske dyrlaegeforening*, **53**, 431.

Nakama, T. (1970) A case of cutaneous alternariosis. *Japanese Journal of Medical Mycology*, **11**, 219.

Neergaard, P. (1945) Danish species of *Alternaria* and *Stemphylium*. Thesis. Munksgaard, Copenhagen and Humphrey Millford, Oxford University Press, London.

Noble, W.C., Rebel, M.H. & Smith, I. (1974) An investigation of the skin flora of dialysis and transplant patients. *British Journal of Dermatology*, **91**, 201.

Park, D. (1968) The ecology of terrestrial fungi. In: *The Fungi* (Ed. by G. C. Ainsworth and A.S. Sussman), Vol. 3, p. 27. Academic Press, New York.

Schlueter, D.P., Fink, J.N. & Hensley, G.T. (1972) Wood-pulp workers disease: A hypersensitivity pneumonitis caused by *Alternaria*. *Annals of Internal Medicine*, **77**, 907.

Takatsuki, Y. (1938) Über eine von einer menschlichen Dermatose isolierte Spezies von *Alternaria*. *The Japanese Journal of Dermatology and Urology*, **43**, 126.

Yu, H. (1965) Studies on fungi of the normal skin. *Acta Dermatologica*, *(Kyoto, Japan)*, **60**, 126.

British Journal of Dermatology (1976) **94**, 211.

Atypical ichthyosiform erythroderma, deafness and keratitis

A REPORT OF TWO CASES

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SUMMARY

Two patients with ichthyosiform erythroderma of the same unusual but characteristic distribution are described. Both patients were born with perceptive deafness and developed severe vascularizing keratitis in early childhood. There is no family history of the disorder in either case. This syndrome is discussed in relation to previous reports of atypical ichthyosiform erythroderma associated with deafness.

Of the various forms of ichthyosis that show an autosomal recessive mode of inheritance, the commonest is non-bullous ichthyosiform erythroderma (Wells & Kerr, 1965). The ichthyosis in this condition is generalized and, though there may be ectropion, there is no associated keratitis or deafness. Other autosomal recessive forms of ichthyosis are very rare and bear little resemblance to our patients. However, there are two case reports (Schnyder, Wissler & Wendt, 1968; Beare *et al.*, 1972) where the ichthyosis had the same striking distribution as in our patients and was associated with deafness. In both these cases there were neurological abnormalities in the lower limbs and physical retardation. Keratitis was not reported in either case.

We are reporting two patients with a similar type of ichthyosis and deafness, but without physical retardation or peripheral neurological abnormality. Their chief clinical problem at the present time is severe vascularizing keratitis which has affected their sight.

CASE REPORTS

Both the patients were referred by consultant dermatologists for investigation and possible treatment.

Both patients were admitted to Guy's Hospital for full clinical and laboratory studies. Immunological investigations were carried out in one because of an increased incidence of infection. Estimates

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of serum vitamin A levels were made because of the reported therapeutic influence of vitamin A on ichthyotic disorders.

Case 1. M.B., male, aged 9 years

He was born, after a full-term pregnancy and a prolonged labour, weighing 3·2 kg. There was no maternal illness during pregnancy. His skin was blue and wrinkled and his face and scalp macerated. By the next morning his skin appeared red and wizened and 5 days later started to peel. A hard crust formed on his scalp and the skin became thickened over his buttocks, the anterior aspect of his lower limbs, his groins, cheeks, scalp and, to a lesser extent, his upper limbs. Erythema became limited to these ichthyotic areas, where it remained visible only at the borders.

He was almost completely deaf from birth and has been helped only minimally by hearing aids.

From birth, his eyelids have been ichthyotic, his conjunctivae have appeared injected and he has been photophobic. At the age of 4 years he developed a corneal ulcer. A plaque, similar to that seen in vitamin A deficiency, was removed from his left cornea at the age of 8 years. It recurred and was removed again 8 months later, leaving him with no sight in his left eye. His right eye has subsequently begun to show the early changes of vascularizing keratitis, in spite of the administration of vitamin A eye drops (Tretinoin 0·025% in arachis oil).

His eyebrows never formed fully and he had no eyelashes at all until his 3rd year of life. Finger nails were present at birth, but toe nails were not seen until he was 2-years-old. His teeth appeared in the normal way, but were brittle and tended to break off and wear down to points.

At 5 months of age he developed large pustules on his scalp, and his finger nails became distorted. He had recurrent bacterial chest infections and episodes of soreness of the mouth, tongue and lips, diagnosed in his 2nd year as oral candidiasis. His finger and toe nails were also found to be infected with Candida albicans. Chest infections tended to be complicated by hyperpyrexia.

He had measles at 18 months, chickenpox when nearly 3-years-old and rubella when aged 7, all without complications except for hyperpyrexia. He has been vaccinated successfully with DPT vaccine.

His growth rate has been normal and he smiled, sat up, crawled, stood up and walked at the same age as his unaffected sibs. His speech has developed to some extent, in spite of his deafness, and he appears to have no intellectual defect. He has been educated partly at home and partly at a school for the deaf.

Family history

A history of skin disease in the family was confined to reports of a nickel sensitivity in his paternal grandmother, a hand eczema in his maternal grandmother and atopic eczema amongst his second cousins. He has two sibs who are neither ichthyotic nor deaf.

His maternal grandmother had six miscarriages following his mother's birth. There is no history of consanguinity.

Physical findings

On examination at the age of 8 years, he weighed 25 kg and was 140 cm tall. Although his whole skin surface was ichthyotic to some extent, there was a striking distribution of more severe ichthyotic areas. These included his cheeks, nose, chin and ears (Fig. 1), his buttocks, groins, the flexor aspects of his forearms and both aspects of his elbows and ankles (Fig. 2). There was crusting and some pustulation over his scalp although this had been considerably improved by treatment with antibiotic ointments. The angles of his mouth and the buccal mucosa showed only minimal signs of candida infection, probably because of the use of oral nystatin preparations. The palms of his hands were hyperkeratotic.

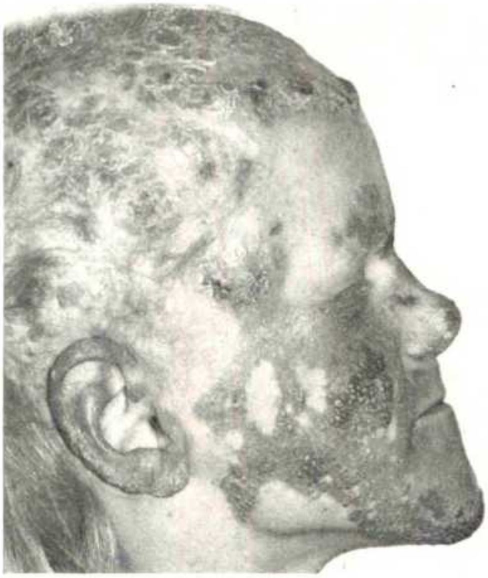

FIGURE 1. Case 1, M.B., face and scalp, aged 8 years.

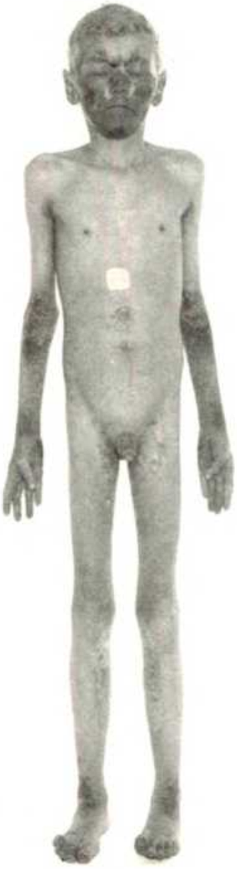

FIGURE 2. Case 1, M.B., aged 8 years.

Examination of the nervous system was within normal limits, except for bilateral nerve deafness and visual loss. Audiograms showed very little hearing present in either ear. Visual loss was complete in the left eye and partial in the right; both his corneae were vascularizing and scarring over. There were no abnormalities in the alimentary, respiratory or cardio-vascular systems.

Investigations

He was investigated primarily for evidence of immunological and vitamin A deficiency. Candida albicans was isolated both from his scalp and from the angles of his mouth. His urine was sterile and a chest X-ray normal. His plasma proteins and immunoglobulins were within normal limits and there was no evidence of auto-antibody formation. He sensitized normally to candidicin and to dinitrochlorobenzene, but not to PPD. A normal percentage of his lymphocytes formed rosettes with sheep red cells. His lymphocytes transformed normally to pokeweed, phytohaemagglutinin and candida. He thus demonstrated normal T-lymphocyte function *in vitro* as well as normal skin sensitization to candida and DNCB *in vivo*.

His serum vitamin A level was within normal limits (100 μg carotenoids/100 ml). The serum iron level was slightly low (60 μg/100 ml), but his total iron binding capacity was within normal limits (274 μg/100 ml).

The following investigations were also within normal limits: electrolytes and urea; calcium; phosphate; alkaline phosphatase; bilirubin; aspartate aminotransferase; fasting glucose; zinc; thyroid function tests; and synacthen stimulation test.

Case 2. P.A., male, aged 20 years

He was born 4 weeks prematurely, after a normal delivery, with a birth weight of 2·75 kg. There was no maternal illness during pregnancy. His skin was normal in colour, but unusually dry, with white 'pin-head' lesions around his nose, while his scalp appeared white and silky. During the 2nd month his mother noticed that his skin had a 'dirty' appearance in spite of regular washing. Throughout the 1st year his skin appeared dry, rough and thickened and, by his 1st birthday, certain well-demarcated areas had become first erythematous and then ichthyotic, leaving them brown and warty with erythematous borders. These changes were restricted to the face and the limbs in a pattern strikingly similar to that of the first case. For 2 weeks, at the age of 3 years, he had widespread 'boils', but blisters were never present at any time.

Complete deafness was noted at the age of 2 weeks and has not been relieved by hearing aids.

His eyes appeared to be normal until he contracted measles at the age of 3, when he became photophobic and his eyes were first noticed to be reddened. Since then his eyes have displayed conjunctival

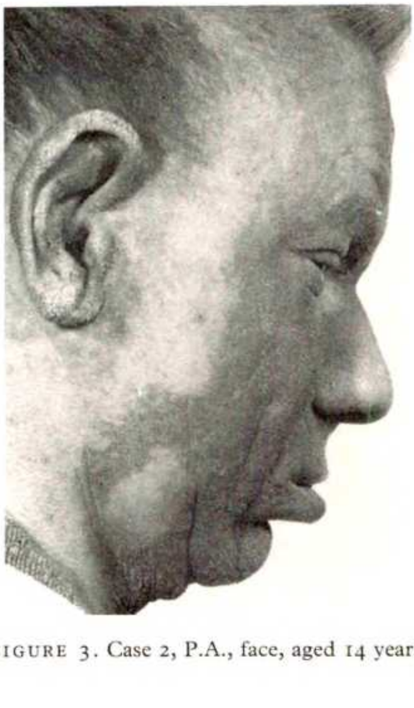

FIGURE 3. Case 2, P.A., face, aged 14 years.

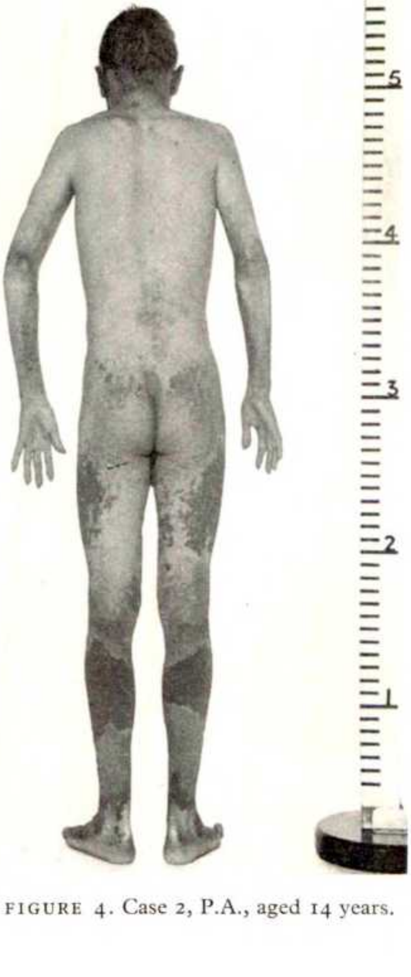

FIGURE 4. Case 2, P.A., aged 14 years.

injection and increased lacrimation associated with keratitis and have often been painful, especially in relation to corneal ulceration. By the age of 10 years a vascularizing keratitis of the left eye had been well established. Examination at the age of 15 years revealed a chronic blepharoconjunctivitis with vascularizing keratitis in both eyes.

His eyebrows were slow to appear and have always been scanty. His eyelashes are sparse and short. Finger and toe nails were not present at birth; his finger nails appeared during the 1st year of life and his toe nails somewhat later. Dentition has been normal.

He has shown no undue tendency to recurrent infections, apart from two episodes of pneumonia between the ages of 13 and 15 years. During each of these illnesses he had a hyperpyrexia.

He had measles, chickenpox and mumps before the age of 5 years, without complications except for hyperpyrexia. He had a tonsillectomy and adenoidectomy at the age of 6 years.

He smiled at the normal age, but was slow to sit up, though he walked at 19 months. He appears to have a normal I.Q., but his speech has developed very poorly, undoubtedly due to his deafness.

Family history

A history of skin disease in the family was limited to his father who had a scaly dermatitis for 2 years which completely recovered. His mother had a partial thyroidectomy at the age of 21 years. He has three older brothers, of whom two are said to be 'educationally backward'. None of his siblings is ichthyotic or deaf. There is no evidence of consanguinity, although three of his four grandparents derive from the same city.

Physical findings

On examination at the age of 19 years his height and weight were within normal limits. The distribution of severe ichthyosiform erythroderma remained essentially the same as at his 1st birthday. His cheeks, nose, chin and ears were involved, with some spread onto the forehead (Fig. 3). His face had taken on a bizarre leonine appearance and felt infiltrated over the forehead. His arms were involved maximally on the flexor surfaces and his legs more circumferentially, especially around the ankle joints (Fig. 4). The palms of his hands showed generalized hyperkeratosis.

His eyes showed a bilateral conjunctivitis and vascularizing keratitis. His vision, in both eyes, was restricted to counting fingers.

He showed complete bilateral perceptive deafness on audiometry, but otherwise no neurological abnormalities. He had bilateral pes cavus. There were no abnormalities in the alimentary, respiratory or cardiac systems; his right testicle was absent and his left testicle atrophic. His I.Q. was estimated as being within the normal range.

Investigations

His urine was sterile and chest X-ray normal. Plasma proteins were normal. Serum IgM and IgG were within normal limits and IgA marginally low (94 mg/100 ml: normal 100–300 mg/100 ml). He sensitized normally to candidicin and to dinitrochlorobenzene, but not to PPD.

His serum vitamin A level was low as measured by serum carotenoids (35 μg/100 ml). The serum iron level was also low (40 μg/100 ml) with a raised total iron binding capacity (495 μg/100 ml).

The following investigations were within normal limits; electrolytes and urea; calcium; phosphate; alkaline phosphatase; bilirubin; aspartate aminotransferase; fasting glucose; thyroid function tests; 3-day faecal fat collection; electrocardiograph and electro-encephalogram.

Treatment and progress

On admission to hospital in September 1974, the best he could do with either eye was to count fingers.

Both corneae showed dense peripheral vascular ingrowth with annular paracentral stromal opacities.

After 3 weeks on vitamin A eye drops and retinoic acid (10 mg four times daily) by mouth the visual acuity with a pinhole was 6/63 in the right eye and 6/60 in the left. Both corneae were much clearer. Prednisolone eye drops were then added to his regime.

After 2 months treatment with vitamin A drops, visual acuity (unaided) improved in the right eye to 6/60 and in the left eye to 6/36.

Histological findings

Skin biopsies from ichthyotic areas on the limbs of both patients showed similar characteristics. The epidermis showed marked hyperkeratosis of a 'basket-weave' pattern, rather than the laminated pattern found in non-bullous ichthyosiform erythroderma. The Malpighian layer was generally of normal thickness but tended to run an undulating course above strikingly elongated, 'peaked', dermal papillae, a pattern uncharacteristic of non-bullous ichthyosiform erythroderma. The granular layer was intact throughout and there were no degenerative or vacuolar changes of the kind seen in bullous ichthyosiform erythroderma. Clear cells were prominent in the basal layer but no excess of melanin was demonstrated.

Cone-shaped keratin plugs were frequently seen in follicular orifices and, in some sections, hyperkeratosis extended into the mouths of eccrine sweat ducts. Sweat duct openings were commonly found at the bottom of the 'valleys' between 'peaked' papillae. Sweat glands were poorly developed, though plentiful in number, and sweat ducts were often thick-walled and somewhat dilated in their middle and upper segments. Inspissated PAS-positive material was identified in the lumina of many of these dilated sweat ducts.

Genetic findings

Both of our patients had unaffected parents and siblings and consanguinity could not be established. Pedigree studies are therefore insufficient to establish the mode of inheritance in this disorder. However, the severity of the condition does suggest an autosomal recessive mode of inheritance.

DISCUSSION

The patients described by Schnyder *et al.* (1968) and Beare *et al.* (1972) closely resembled each other and each of our two patients. The distribution of ichthyosiform erythroderma over circumscribed areas of the face, buttocks and limbs is common to all four patients, each of whom also has complete or sub-total bilateral perceptive deafness.

The two previously-reported patients showed two other similarities in that both were physically retarded and both had bilateral talipes deformities. In the case of Beare *et al.* (1972) a neuropathy associated with this disorder was also found, but not in the case of Schnyder *et al.* (1968). Neither of our two patients is physically retarded and only Case 2 has a bilateral talipes deformity which, as in Schnyder's case, is not associated with a neuropathy.

There were other differences between the two previously reported cases besides the presence or absence of a neuropathy. The first was reported as being mentally retarded, although repeated estimates of I.Q. as the patient grew older seemed to be approaching closer to normality (81, aged 10 years), whereas the patient of Beare *et al.* (1972) had a normal I.Q. at the age of 8 years. Neither of our patients is mentally retarded. Schnyder *et al.* (1968) reported a reduced serum level of vitamin A whereas Beare *et al.* (1972) found normal levels in their case. Our first patient has normal levels, whereas our second has reduced levels.

Neither of the previously reported patients was described as having a bilateral vascularizing keratitis, but in both of our patients this was the main clinical problem in later childhood. The efficacy of treating this complication with vitamin A eye drops has been as striking in one patient as it has been disappointing in the other. It should be noted that our estimates of serum vitamin A showed a reduced level in the responsive case as opposed to a normal level in the unresponsive.

The apparently increased susceptibility to infection of our first patient remains unexplained in the face of normal immunological findings. It is a feature shared neither by our second patient nor by the two previously reported patients.

Histological findings were different in both our cases from either bullous or non-bullous ichthyosiform erythroderma. Beare *et al.* (1972) also reported a 'basket-weave' hyperkeratosis and Schnyder *et al.* (1968) described the appearance of the base of the epidermis as 'saw-toothed', corresponding to our description of 'peaked' papillae.

We consider that the four cases share enough important similarities to be included in the same syndrome of atypical ichthyosiform erythroderma associated with deafness. Keratitis, physical retardation and peripheral neuropathy appear to be variable additional associations.

ACKNOWLEDGMENTS

Our thanks to Dr Brian Russell for referring the first patient to us to study, to Professor Edward Wilson Jones for his help with the histological studies, and to the Herbert E. Dunhill Trust for a generous grant to help with the investigation of these patients.

REFERENCES

BEARE, MARTIN J., NEVIN, N.C., FROGGATT, P., KERNOHAN, D.C. & ALLEN, INGRID, V. (1972) Atypical erythrokeratoderma with deafness, physical retardation and peripheral neuropathy. *British Journal of Dermatology*, **87**, 308.

SCHNYDER, U.W., WISSLER, H. & WENDT, G.G. (1968) Eine weitere Form von atypischer Erythrokeratodermie mit Schwerhörigkeit und cerebraler Schädigung. *Helvetica Paediatrica Acta*, **23**, 220.

WELLS, R.S. & KERR, C.B. (1965) Genetic classification of ichthyosis. *Archives of Dermatology*, **92**, 1.

British Journal of Dermatology (1976) **94,** 219.

Brief Communications

Acid hydrolases in psoriatic epidermis

P.D.Mier and José J.M.A. van den Hurk *Department of Dermatology, University of Nijmegen, The Netherlands*

This laboratory is currently investigating the levels of acid hydrolase activity in the epidermis of various skin lesions, and our findings will be published in full in due course. The results which we have obtained for psoriasis, however, seem sufficiently remarkable to warrant preliminary publication.

Epidermal slices were cut using a keratotome set for a depth of 0·2 mm, frozen in liquid nitrogen, and stored at −20°C prior to analysis. Specimens were homogenized at 20 mg/ml in bovine serum albumin (1 mg/ml), centrifuged, and the activity of fourteen acid hydrolases determined in the supernatant using techniques already described (Mier & van den Hurk, 1975a–c).

The activity of each enzyme in eight specimens of psoriatic lesion, seven specimens of uninvolved epidermis from psoriatic patients, and thirty-six specimens from healthy controls is shown in Table 1. Although there is a tendency for all enzymes to be increased in the lesion (suggesting a greater number of lysosomes in comparison to normal epidermis), it is clear that there are striking and specific increases in the levels of arylsulphatases A and B and of β-glucosidase. These enzymes are increased 34-fold, 22-fold and 11-fold over the respective mean control values; there is no overlap with the normal range. The values for the uninvolved psoriatic epidermis, by contrast, are identical to the controls.

Since lysosomal hydrolases are (at least in some tissues) inducible enzymes, these findings point to the accumulation of a metabolite which contains β-glucoside residues and which is sulphated. The most likely candidate, on the rather scanty evidence currently available, seems to be a sulphated glycolipid. These compounds are characteristically located in the plasma membrane, where they may be associated with membrane-bound enzymes (Karlsson, Samuelsson & Steen, 1974). Thus our findings are consistent with, and may be causally related to the defects which have been described in adenyl cyclase (Hsia *et al.*, 1972; Wright *et al.*, 1973; Yoshikawa *et al.*, 1975; Mui, Hsia & Halprin, 1975) and in the membrane-bound ATP hydrolytic activity (Mahrle & Orfanos, 1974) of the psoriatic lesion.

The authors wish to thank Professor J.W.H.Mali and the medical staff of this Department for their help in providing the clinical material used in this work.

REFERENCES

Hsia, S.L., Wright, R., Mandy, S.H. & Halprin, K.M. (1972) Adenyl cyclase in normal and psoriatic skin. *Journal of Investigative Dermatology*, **59,** 109.

Karlsson, K.-A., Samuelsson, B.E. & Steen, G.O. (1974) The lipid composition and Na^+-K^+ dependent ATPase activity of the salt (nasal) gland of eider duck and herring gull: a role for sulphatides in sodium-ion transport. *European Journal of Biochemistry*, **46,** 243.

Mahrle, G. & Orfanos, C.E. (1974) Decreased membrane-bound ATP-hydrolytic activity in psoriatic epidermis. *British Journal of Dermatology*, **91,** 529.

Mier, P.D. & van den Hurk, J.J.M.A. (1975a) Lysosomal hydrolases of the epidermis. 1. Glycosidases. *British Journal of Dermatology*, **93,** 1. *Idem* (1975b) 2. Ester hydrolases, **93,** 391. *Idem* (1975c) 3. Peptide hydrolases, **93,** 509.

Mui, M.M., Hsia, S.L. & Halprin, K.M. (1975) Further studies on adenyl cyclase in psoriasis. *British Journal of Dermatology*, **92,** 255.

Wright, R.K., Mandy, S.H., Halprin, K.M. & Hsia, S.L. (1973) Defects and deficiency of adenyl cyclase in psoriatic skin. *Archives of Dermatology*, **107,** 47.

Yoshikawa, K., Adachi, K., Halprin, K.M. & Levine, V. (1975) On the lack of response to catacholamine stimulation by the adenyl cyclase system in psoriatic lesions. *British Journal of Dermatology*, **92,** 619.

TABLE I. Acid hydrolase activities in normal and psoriatic epidermis

	α-glu	β-glu	α-gal	β-gal	α-man	β-NAG	β-gln	AP	sf-A	sf-B	cat B_1	cat C	cat D	aram
Controls (36)														
Highest	21	3·8	17	67	64	450	150	1900	1·5	1·1	11	130	3·5	610
Mean	11	1·3	9·9	38	29	130	46	810	0·47	0·43	5·6	59	1·7	290
Lowest	2·9	0·14	5·0	8·9	16	32	5·1	280	0·07	0·05	1·8	28	0·80	85
Psoriasis														
Lesion 1	23	15	13	66	40	180	100	2000	18	11	6·4	150	3·5	860
Lesion 2	24	16	23	83	48	230	90	3300	22	15	6·0	93	4·8	940
Lesion 3	15	6·5	9·2	46	38	150	66	2100	8·9	4·2	5·1	90	5·4	690
Lesion 4	23	9·5	23	43	33	150	89	2600	15	7·9	8·1	180	6·6	1100
Lesion 5	21	12	15	66	41	160	76	2100	18	11	6·5	150	7·1	870
Lesion 6	19	13	11	42	33	130	56	1300	8·3	4·6	5·6	78	6·0	970
Lesion 7	16	21	12	47	31	160	95	1900	12	7·1	5·6	81	8·9	840
Lesion 8	30	22	20	73	29	210	89	2400	28	14	7·1	150	7·8	990
'Uninvolved' (mean of 7)	11	1·5	11	39	26	100	50	900	0·42	0·48	5·0	50	1·9	300

Values are in nmol/min/g wet weight. Abbreviations are: glu = glucosidase, gal = galactosidase, man = mannosidase, NAG = N-acetylglucosaminidase, gln = glucuronidase, AP = acid phosphatase, sf = sulphatase, cat = cathepsin, aram = arylamidase.

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Mycosis fungoides: model for T-lymphocyte homing to the skin?

M. Goos, E. Kaiserling and K. Lennert

Departments of Dermatology and Pathology, University of Kiel, Germany

Up to the present day there has been no satisfactory explanation of why benign or malignant lymphomas can develop in the skin. Recently Goudie, MacFarlane & Lindsay (1974) presented an interesting hypothesis concerning the homing of lymphocytes to non-lymphoid tissues. The authors speculated that the proliferation of extranodal lymphoid tumours might be due to the possibility that neoplastic lymphocytes home on a special tissue by a process called ecotaxis (de Sousa, 1971). Ecotaxis means the accumulation of lymphocytes in territories which display their appropriate microenvironment.

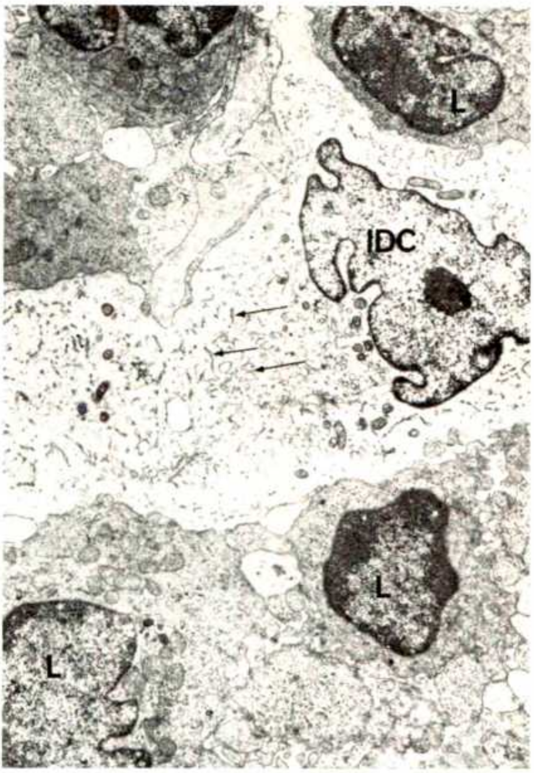

FIGURE 1. Mycosis fungoides of the skin: interdigitating reticulum cell (IDC) in close contact with lymphatic tumour cells (L). Arrows indicate the tubulo-vesicular system (× 6600).

Reprint requests to: Dr Manfred Goos, D-23 Kiel, Schittenhelmstraße 7, Germany.

Our investigations on mycosis fungoides revealed that in the skin a specific reticulum cell may be found which resembles the interdigitating reticulum cell (IDC) of T-cell areas in the lymphoid tissues (Veldman, 1970). IDC can only with difficulty be found in light-microscopic sections. In the electron microscope they have a peculiar appearance. They are characterized by an electron-translucent cytoplasm with numerous vesicles as well as short profiles of rough and smooth endoplasmic reticulum (tubulo-vesicular system). The cytoplasm has long-branched processes which are in close contact with neighbouring lymphocytes of small and medium size, blast cells and IDCs (Fig. 1).

The occurrence of IDC in the skin not only provides evidence of a T-cell specific microenvironment in mycosis fungoides (Goos, Kaiserling & Lennert, in preparation) and supports its T-cell nature (Edelson *et al.*, 1974), but also may be the morphological basis of T-lymphocyte homing to the skin.

ACKNOWLEDGMENT

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REFERENCES

EDELSON, R.L., KIRKPATRICK, C.H., SHEVACH, E.M., SHEIN, P.S., SMITH, R.W., GREEN, I. & LUTZNER, M. (1974) Preferential cutaneous infiltration by neoplastic thymus-derived lymphocytes. *Annals of Internal Medicine*, **80**, 685.

GOOS, M., KAISERLING, E. & LENNERT, K. (1976) T-cell-specific microenvironment in mycosis fungoides of the skin. *Virchows Archiv für pathologische Anatomie und Physiologie* (in preparation).

GOUDIE, R.B., MACFARLANE, P.S. & LINDSAY, M.K. (1974) Homing of lymphocytes to non-lymphoid tissues. *Lancet*, **i**, 292.

SOUSA, M.DE (1971) Kinetics of the distribution of thymus and marrow cells in the peripheral lymphoid organs of the mouse: ecotaxis. *Clinical and experimental Immunology*, **9**, 371.

VELDMAN, J.E. (1970) *Histophysiology and electron microscopy of the immune response.* Habilitationsschrift, Groningen.

British Journal of Dermatology (1976) **94**, 223.

Review

Tropical dermatology

CUTANEOUS LEISHMANIASIS

This review does not cover the whole field of cutaneous leishmaniasis, nor discuss recent experimental and immunological work. It is intended, however, to remind clinicians of the extent of this common disease, of its various clinical presentations and of the problems of diagnosis and treatment which are still with us.

Geography

The disease is present in the old world throughout the Mediterranean countries, the Middle East and Central Asia. In Africa it is found around the edge of the Sahara, including the Savanna belt of West Africa, and in the highlands of Ethiopia and Kenya. South of this it is extremely rare.

In the new world it exists in all central and northern South America, including the western slopes of the Peruvian Andes, and especially in the Amazonian Forest, where the agent *Leishmania brasiliensis brasiliensis* is responsible for the severely mutilating late metastatic lesions of the nose and mouth, known as espundia.

Clinical features

Most physicians are now familiar with the four stages of a classical case of cutaneous leishmaniasis: (1) a painless nodule, which (2) crusts centrally and may ulcerate before (3) starting to heal leaving (4) a depressed mottled scar. The nodule is distinguished from a slowly healing insect bite by its steady growth to 1 cm or more; it does not itch. The second stage is distinguished from a simple chronic boil by its nodular edge and, very often, satellite nodules. The rural zoonotic Mediterranean, Middle Eastern and West African varieties go through these stages in a few weeks or months, often with much surrounding allergic inflammation and lymphadenitis. West African lesions are commonly

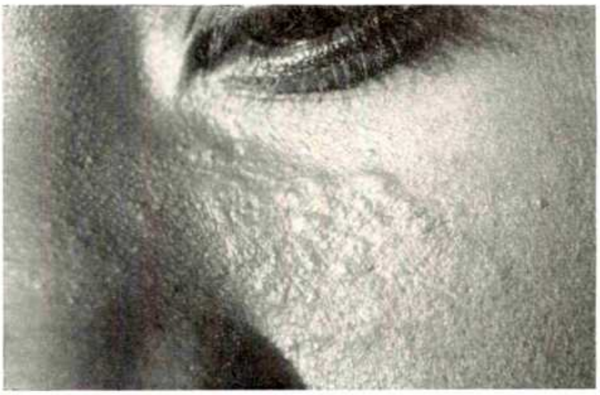

FIGURE 1. Solitary early micropapular lesion on the cheek of an Ethiopian boy. Parasites readily demonstrable, intermediate type of histology (Bryceson, 1970), weakly positive leishmanin test.

on the limbs, multiple and greatly crusted. Urban Middle Eastern and especially Ethiopian lesions may take up to 2 years to heal and spend a long time in the first and second stages, with no added inflammation.

In Ethiopia and Kenya the initial nodule may, rarely, never ulcerate but spread locally to form multiple nodules or a large plaque. Commonest over the face, this can superficially resemble lupus erythematosus but there is no follicular plugging. Months or years later when metastatic lesions appear on face, ears and extensor surfaces of the limbs, this condition of diffuse cutaneous leishmaniasis can mimic lepromatous leprosy, but there is no nerve damage (Bryceson, 1970).

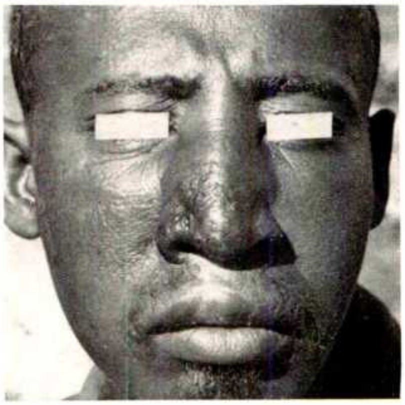

FIGURE 2. Chronic mucocutaneous leishmaniasis in an Ethiopian, clinically similar to espundia. Note expansion of nose and deformity of right ala. Mucosal skin and cartilage infiltrated with tuberculoid granulomata. Parasites difficult to demonstrate. Marked hypersensitivity to leishmanin.

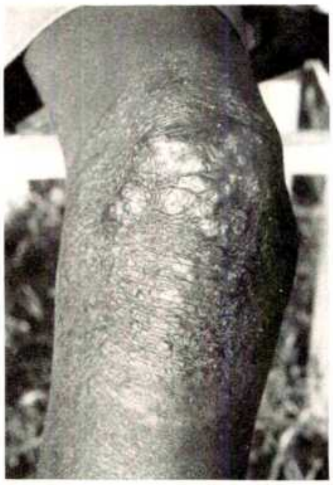

FIGURE 3. One of several similar lesions, characterized by a large macule of altered skin texture but normal colour and sensation, and containing many papules some of which have crusted or ulcerated. Parasites demonstrable in smears and with difficulty on histology, which was intermediate. Weakly positive leishmanin test.

Relapsing or recidivans leishmaniasis presents a typical picture of a scar around which a succession of nodules, often caseating, emerge and heal slowly. Rarely there may be an advancing edge and a healing tail as with lupus vulgaris. The cases are usually from Middle Eastern towns, but have also been seen following vaccination with a live strain of *Leishmania*, as is practised in several countries including U.S.S.R., Israel and Iraq.

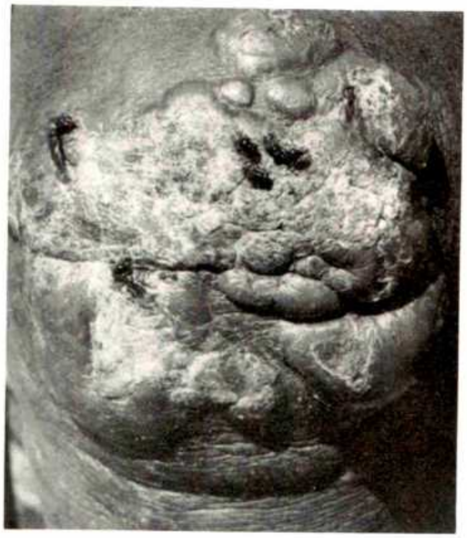

FIGURE 4. Diffuse cutaneous leishmaniasis on the knee of an Ethiopian. This large multinodular lesion contained innumerable parasites shown histologically to be inside vacuolated macrophages, with no surrounding lymphocytic response. Ulceration is probably secondary to trauma in this instance. Leishmanin negative.

In South and Central America the great majority of lesions behave in the classical manner. Mexican lesions (caused by *L. mexicana*) are especially common on the ear where they may linger for many years. Lesions due to *L. brasiliensis brasiliensis* can produce deep 'punched-out' ulcers on the limbs with very little nodulation.

Diagnosis

The best, simplest and quickest method of diagnosis is the slit skin smear. The skin of a nodule (main or satellite) is slit with a scalpel blade to a depth of 1–2 mm and the cut surface scraped and smeared onto a clean microscope slide, which is dried and stained with Leishman's stain and examined for amastigotes. A bloody smear is valueless. If there is no suitable nodule, or the nodule on incision proves to be caseous, it is necessary to clear out the crust and slough down to the ulcer bed and scrape here. Pus and debris seldom contain identifiable parasites. About 60% of lesions in first and second stages can be confirmed in this way (Lemma *et al.*, 1970).

Material obtained by scraping should also be inoculated to NNN medium which increases the diagnostic yield to over 90%. The whole volume of overlay fluid of a negative culture should be subcultured after 1 week and re-examined weekly for 4 weeks.

Biopsy is third best, parasites are difficult to find and the granuloma by itself is never diagnostic. If a biopsy is taken, impression smears of its cut surface should be made, before the tissue is fixed.

The leishmanin skin test is analagous to the mantoux test in tuberculosis. A semi-purified protein extract of cultured promastigotes can be standardized to produce a satisfactory, quantified reagent but is not generally available. The test is a useful diagnostic aid in a European tourist but must be cautiously interpreted in a native of an endemic area where the prevalence of sensitivity in the adult population may exceed 90%.

Serology has little or no place in diagnosis although antibody may be detectable by fluorescence in cases where there is marked lymph-node involvement and in cases of espundia (Ranque & Quilici, 1970).

Treatment

The disease is usually self-healing and scarring is inevitable, either with or without treatment, once the second stage is reached. Toxic drugs are therefore contraindicated and local treatment which will make scarring worse should be avoided. Most cases of simple cutaneous leishmaniasis strictly do not need treating except perhaps to reduce the healing time of a lesion, especially in a disfiguring site. Amazonian lesions should always be treated because of the risk of espundia.

There is still no satisfactory simple treatment for cutaneous leishmaniasis. The best drug of a bad bunch remains pentavalent antimony (sodium stibogluconate 'Pentostam', 'Glucantine') which is given in a dose of 0·1 ml of a 34% solution, per kg body weight, daily for at least 14 days, by intramuscular or preferably intravenous injection. The drug is virtually non-toxic in this dose, but when given intravenously the patient's cardiac rhythm and systemic blood pressure should be observed carefully for the next hour. Vomiting or an arrhythmia call for a pause in treatment. The Ethiopian and Kenyan parasite is resistant to antimony and the diffuse disease may require one of the second line drugs such as pentamidine or amphotericin B, both of which are seriously toxic and should not be handled by the inexperienced. The additional use of systemic or local steroids is said to be useful in healing recidivans leishmaniasis, but no formal study has been conducted.

Local infiltrations are not to be recommended, but cryotherapy and even radiotherapy have their advocates. Local heat, as coned infrared rays, has proved effective in Iraq and is safe but time consuming.

Several other drugs have been claimed as useful but have not stood the test of time. Metronidazole and antimalarials are without effect. Rifampicin is the latest for which success has recently been claimed, but a formal trial has yet to be carried out. In cutaneous leishmaniasis, as in leprosy, drug trials are very difficult to perform satisfactorily because the disease is almost invariably self-healing.

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REFERENCES

BRYCESON, A.D.M. (1970) Diffuse cutaneous leishmaniasis in Ethiopia. *Transactions of the Royal Society of Tropical Medicine and Hygeine*, **63**, 108.

LEMMA, A., FOSTER, W.A., GEMETCHU, T., PRESTON, P. M., BRYCESON, A. & MINTER, D.M. (1969) Studies on leishmaniasis in Ethiopia. *Annals of Tropical Medicine and Parasites*, **63**, 435.

RANQUE, J. & QUILICI, M. (1970) Recent advances in immunodiagnosis of leishmaniasis. *Journal of Parasitology*, **56**, 277.

British Journal of Dermatology (1976) **94**, 227.

Society Proceedings

South West of England and Wales Dermatological Society

8 February 1975, Bristol Royal Infirmary

Chairman: Dr R.P.WARIN

Pressure urticaria in father and son. DR R.P.WARIN

A man, aged 38 years.

History. Five years ago, the patient suddenly developed unexplained angio-oedema of his face; within 2 days this settled, but it was followed by chronic urticaria which has continued to the present time, producing a few weals most days. A test dose of aspirin (300 mg) caused an exacerbation.

Since the onset, he has noticed swellings at the site of pressure. These occur 2–4 h after the pressure and last for 1–2 days. They have developed on the hands, feet, back, shoulders and elbows—in fact, anywhere subjected to continuous pressure. He is now unable to carry out any manual work.

Family history. His 2-year-old son started to develop swellings at the site of pressure 6 months ago. These were first noticed over the buttocks after riding a new tricycle.

There are three older children. Neither they nor any other members of the family have a similar condition.

On examination. There were a few scattered chronic urticaria weals. Pressure tests had been applied (7 kg weight for 5 min) on the right forearm 12 h before the meeting, and on the left forearm 2 h before the meeting. Well-marked swellings were present at both sites, the larger on the right.

The son was not shown at the meeting, but a similar pressure test had recently been done on him and had produced a well-marked swelling. He also had a localized patch, 3 cm in diameter, on the centre of his back, typical of urticaria pigmentosa.

Investigations. The serum C1-esterase inhibitor level and the euglobulin lysis time were normal.

Treatment. Prednisone, 20 mg daily by mouth, had helped but had produced severe systemic steroid effects at this dosage; smaller dosage was ineffective.

Antihistamines and hydroxyzine had no effect on the pressure weals but reduced the chronic urticaria.

Phenformin and ethylestrenol were not effective, and Trasylol given intravenously only temporarily reduced the pressure effect.

Comment. Pressure urticaria in patients with chronic urticaria has previously been described; for example, four of the fifteen patients with pressure urticaria reported by Ryan, Shim-Young & Turk (1968) had chronic urticaria, and one of them had an exacerbation after taking aspirin. There seems to be no previous report of any family incidence of pressure urticaria.

It is interesting to speculate whether the localized patch of urticaria pigmentosa in the son might be related to the pressure urticaria, but the association does not seem to have been described before.

REFERENCE

RYAN, T.J., SHIM-YOUNG, N. & TURK, J.L. (1968) Delayed pressure urticaria. *British Journal of Dermatology*, **80**, 485.

Ashy dermatosis. DR R.D.G.PEACHEY

A girl, aged 16 years.

History. In March 1971, she was immunized against rubella, and in April of the same year she began to notice a mottled pigmentation appearing on her neck, trunk, and proximal parts of her limbs (Fig. 1).

No erythematous rash had been noticed prior to the development of the pigmentary changes. She had had no serious illness in the past, and had not been taking any medication. She had always tanned readily after exposure to sunshine.

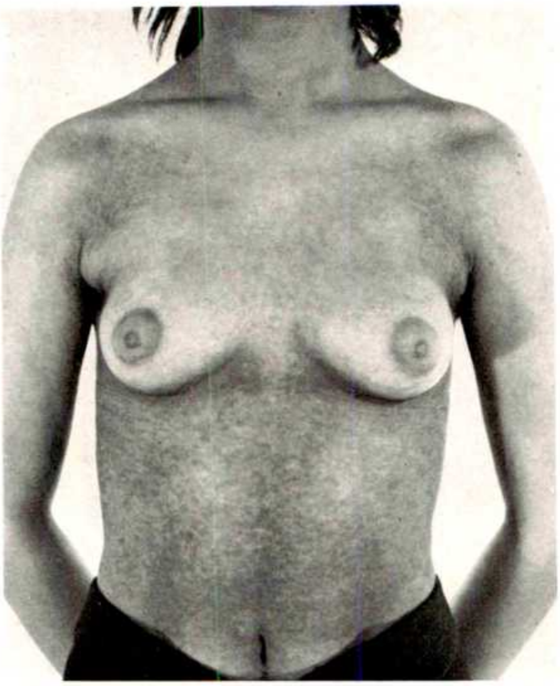

FIGURE 1. Ashy dermatosis.

The pigmentary changes have remained essentially unchanged since 1971.

On examination. There was a mottled and reticulate slate-coloured pigmentation on the neck, trunk, and the proximal parts of the limbs.

Investigations. Histologically, the affected skin showed small groups of melanin-laden histiocytes in the dermis, and a few lymphocytes scattered around some of the dermal blood vessels.

Treatment. Topical applications of hydroquinone 5–15% in a cream base were not beneficial, but cosmetic camouflage has been helpful.

Comment. In 1957, Ramirez described several patients with patches of greyish cutaneous discolouration of unknown aetiology; he called the condition dermatosis cenicienta (ash-coloured dermatosis). In a further report (Ramirez, 1966), he stated that by 1965 a total of 139 cases had been discovered mainly in El-Salvador, but some in Venezuela and other Latin American countries and in the U.S.A.

In recent years, further similar cases have been reported (Knox, Dodge & Freeman, 1968), and occasional patients have been shown at clinical meetings in Britain.

The patient reported here is of English stock, and has never been abroad.

REFERENCES

KNOX, J.M., DODGE, B.G. & FREEMAN, R.G. (1968) Erythema dyschromicum perstans. *Archives of Dermatology*, **97**, 262.

RAMIREZ, C.O. (1957) Los cenicientos. Problema clinico. *Proceedings of the First Central American Congress of Dermatology, San Salvador, 5–8 December, 1957*, p. 122.

RAMIREZ, C.O. (1966) Dermatosis cenicienta. Estudio epidemiologico de 139 casos. *Dermatologia (Revista Mexicana)*, **10**, 133.

British Journal of Dermatology (1976) **94**, 229.

Book Review

Pocket Color Atlas of Dermatology. JOSEPH KIMMIG and MICHAEL JANNER (1975) American edition translated and revised by HERBERT GOLDSCHMIDT. Stuttgart: Georg Thieme. Pp. 218, 302 figures (in colour).

In recent years two new colour atlases, intended as introductions to dermatological diagnosis, have appeared in Great Britain, the Wolfe volume by Levene and Calnan and the Update publication by Fry, both in hard back. Their hegemony is unlikely to be challenged by the new paperback edition of the Atlas of Kimmig and Janner of Hamburg in an American edition translated and revised by the Philadelphian dermatologist, Herbert Goldschmidt.

Many of the colour photographs are good but others are not sufficiently close up to be useful, e.g. those of lichen planus. The text is accurate but no distinction is made between common and rare conditions which are often accorded equal amounts of pictorial and textual space. The beginner can thus gain no sense of diagnostic probability from this volume alone.

Little attempt has been made in this edition to bring it up to date. For instance, the excessively rare congenital erythropoietic porphyria is illustrated but the not uncommon erythropoietic protoporphyria is missing, as is, for example, peri-oral dermatitis. There are twenty-five pictures of syphilis but the important and characteristic cutaneous pustulosis of gonococcal septicaemia is absent. The section on bullous diseases illustrates rare pemphigus vegetans but bullous pemphigoid is missing, although mentioned in the text. The account of dermatitis herpetiformis fails to mention the gut. This little book's collection of pictures perhaps deserves a place in skin department libraries but it cannot be recommended to undergraduates or M.R.C.P. candidates.

HARVEY BAKER

Sarcoidosis. A Clinical Approach. O.M.P. SHARMA (1975) Springfield Illinois, Charles C. Thomas. Pp. 68, illustrations, 5 plates. Price $17·50.

The author, who is Associate Professor of Medicine at the University of California School of Medicine, has modestly set out to present a 'concise and practical monograph' for those who have 'limited contact with the disease'. He has done just that. This is a clear and well-written account which follows the traditional pattern of clinical and pathological presentations and complications of this curious and pervasive disease. The section on cutaneous aspects is straightforward and emphasizes the rather bizarre and unusual form seen in the negro. He has added a chapter on sacroidosis in 'special situations', e.g. in childhood, old age and with infections, malignancy and auto-immune disease; this might well have been longer.

In view of the continuing mystery of the aetiology of sarcoidosis, it is not surprising that Dr Sharma has little new to offer from his own experience of 150 cases (from which he draws liberally to illustrate the text). Most dermatologists in this country see this disease fairly often and Scadding's standard work will probably remain their 'Bible'. But it is much less common outside north-west Europe. The 471 references given from the last 4 years are comprehensive and valuable and this short book gives a useful and broad introduction to the subject. No-one will go far wrong in diagnosis and treatment if he follows its precepts.

D.S.WILKINSON

Simulations in Dermatology. Selected Problems in Patient Management. DAVID L. RAMSAY and LAWRENCE M. SOLOMON (1975) Appleton-Century-Crofts, New York: Pp. 203. illustrated in colour. Price £10.20 (Paper).

This book is an attempt to simulate specific problems in clinical dermatology. Each problem is illustrated by a colour print, in most cases of such poor quality that it would be best omitted, and then a questionnaire. The answer to each question can be revealed by rubbing the appropriate space with the special pencil provided.

As an example, Judy who is illustrated by a colour print of her shins which shows nothing of clinical significance, is described as having painful nodules of the lower part of both legs. Then follows a long series of questions, investigations and treatment procedures, in all 183. Many of these are irrelevant but this is intentional in order to make it more difficult to come to the correct conclusion and to simulate the situation in real life.

This kind of alternative to learning medicine in the clinic or at the bedside is not very effective, although it may furnish an intellectual exercise and give the student some idea of what he may have failed to learn. The structure of the questionnaires is valid but there should be some way of indicating the correct way of arriving at the right answer with the minimum number of answers and investigations. For instance in Judy's case, as she had sarcoid I do not think that as a routine DNCB sensitization should be attempted, although the answer shows that it was impossible to sensitize the patient. The chest X-ray changes, the positive Kveim test and the infiltration of a Caesarean section scar associated with erythema nodosum would, I think, be sufficient to get the correct diagnosis. Extra marks should be given for investigating those systems which might be involved in sarcoidosis, such as the eyes and the nervous system.

I cannot see this type of book becoming popular. Once the book has been used, it has lost its value except for the short discussion of each problem in the appendices.

R.H.MEARA

Korrektive Dermatologie. J.PETRES and M.HUNDEIKER (1975) Berlin, Heidelberg and New York: Springer. Pp. 135, 178 illustrations.

The Germans have the not altogether undeserved reputation of writing exhaustively if not exhaustingly on any given subject. This little book, however, is brief and clear, yet comprehensive and well documented. In less than 100 pages of text, which includes 84 simple line-drawings, the authors cover the use of operative procedures for lesions of the skin—these range from simple curettage to major plastic reconstructions.

An introductory section of 30 pages considers the indications for treatment; the next 15 pages describe general principles and techniques and the concluding 43 pages of text deal with procedures in special areas—scalp, nose, eyelid, etc. There are 520 references to papers in the world literature and the book ends with 21 pages of 'before, during and after' photographs of patients subjected to some of the operations described.

Clearly the range of procedures is much wider than the average British dermatologist would wish to undertake himself, nor is he likely to be qualified to do so. Nevertheless, an understanding of operative possibilities is of great value in advising patients about treatment.

Very little in this field has been written specifically for dermatologists (E.EPSTEIN: *Skin Surgery*, 3rd edition, 1971; R.L.BAER & A.W.KOPF: *Dermatologic Office Surgery* in *Yearbook of Dermatology* 1963–64; D.S.WILKINSON: *Physical and Surgical Procedures*, Chapter 70 of *Textbook of Dermatology* by ROOK, WILKINSON & EBLING, 2nd edition, 1972).

I know of nothing as handy as this German book and it is a pity that the language will prevent its wide use by our monoglots.

P.J.HARE

Acne. Morphogenesis and Treatment. G. PLEWIG and A.M. KLIGMAN (1975) Berlin: Springer. Pp. 333, 110 illustrations, mostly colour. Price $50.40

Some dermatological books appeal to the reader by virtue of the clarity of their text; others by the standard of the pictures. Plewing and Kligman introduce their personalized monograph by stating that one picture is better than a thousand words. This is certainly a valid comment. The standard of the pictures, both clinical and experimental, is outstanding. The pictures take the reader logically through the function of the pilo-sebaceous unit, the pilo-sebaceous unit in disease and the effect of some, at times maybe biased, treatments of acne. The authors comment that their written account is meant to be kept to a minimum. Despite this the limited text gives a concise objective review of the problem, clinically, experimentally and therapeutically. Plewig and Kligman have integrated their own work with many of the important references. This work is to be recommended as a must for the libraries of all dermatology departments. It is also a must for anyone interested in acne and will be of help to our colleagues in the pharmaceutical industry.

W.J.CUNLIFFE

British Journal of Dermatology (1976) 94, 232.

News and Notices

International Symposium on Occupational Dermatology

An international Symposium on Occupational Dermatology will be held at the Free University of Amsterdam on 29 May 1976, under the auspices of the Dutch Society of Dermatologists. Speakers are: H. Schäfer, Berlin; H. Maibach, San Francisco; J.M. Lachapelle, Brussels; S. Fregert, Lund; J. Nater, Groningen; W. van Ketel, Amsterdam; K. Malten, Nijmegen. Basic information will be sent to early registered participants and may be obtained from: Professor Dr K.E. Malten, Section Occupational Dermatology, Dermatology Department of the Catholic University, Javastraat 104, Nijmegen, The Netherlands.

APPOINTMENTS

Yorkshire Regional Health Authority

Dr Ronald W.B. Scutt has been appointed Consultant Dermatologist to the Scunthorpe General Hospital, Yorkshire Regional Health Authority. Dr Scutt qualified M.B., B.S., London, in 1939, having trained at the Westminster Medical School. He had various Dermatological Registrarships in Bristol, Norwich and at the Westminster and St John's Hospitals, London, from 1946 to 1951. He then joined the Royal Navy where he served as adviser on Dermatology, being based in the Portsmouth area and he retired in the rank of Surgeon Captain. In 1973 he was Medical Adviser to Fisons Pharmaceuticals in the Middle East. He has written on various topics concerning Contact Dermatitis but notably on the subject of tattoos and is joint Author of a textbook on the subject.

University of London

The title of Professor of Dermatology has been conferred on Dr P.F.D. Naylor in respect of his post at St Thomas's Hospital Medical School.

Professor Naylor received his medical education at Gonville and Caius College, Cambridge, where he took two firsts, and St Thomas's Hospital Medical School, qualifying M.B., B.Chir. in 1948. Subsequently, he became house physician to Professor Sharpey-Schafer before joining the Army where he worked in the Physiology section of the Army Operational Research Group. There he did research on load-carrying and also on the effects of friction on the skin. After leaving the Army, the work on friction was continued as a Research Fellow to the Institute of Dermatology and enabled him to write his M.D. Thesis on this topic before joining the Medical Unit at St Thomas's as a lecturer in 1955. At this time he became interested in the development and use of polarographic methods for measuring oxygen tension in tissues and received the Polarographic Society Award for this work in 1960. In 1957 he became Senior Lecturer in Dermatology at St Thomas's and collaborated with Dr Dowling in research on the mechanisms of alkali neutralization by the skin which they published jointly. In 1962 he became an honorary consultant to the Department of Dermatology, St Thomas's Hospital and in 1967, Titular Reader in Dermatology in the Medical School. In 1971 he was also appointed Adviser in Clinical Studies, St Thomas's Hospital Medical School. In recent years his chief research interest has been in the skin microcirculation and oxygen transport and diffusion.

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
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milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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1. Bunney, Mary H. (1971), Practitioner, **207**, 197.
2. Pegum, J. S. (1972), Practitioner, **209**, 453.
3. Bunney, Mary H. (1974), Prescribers Journal, **14**, 118.

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Walter F. Lever M.D. and Gundula Schaumburg-Lever M.D. *Fifth Edition*, 1975. 816 pages, 436 illustrations (8 colour). Lippincott, £36.40

In the eight years that have passed since publication of the fourth edition so much new information has become available on nearly every major dermatosis that the book had to be entirely rewritten. The greatest changes were required in the description of the lymphomas because they have been reclassified in recent years. Many changes have been made also in the description of the bullous dermatoses and of the metabolic diseases, especially of amyloidosis, colloid milium, hyalinosis cutis et mucosae, and porphyria. Also the division of malignant melanoma in situ into two types with different prognosis has been given due consideration.

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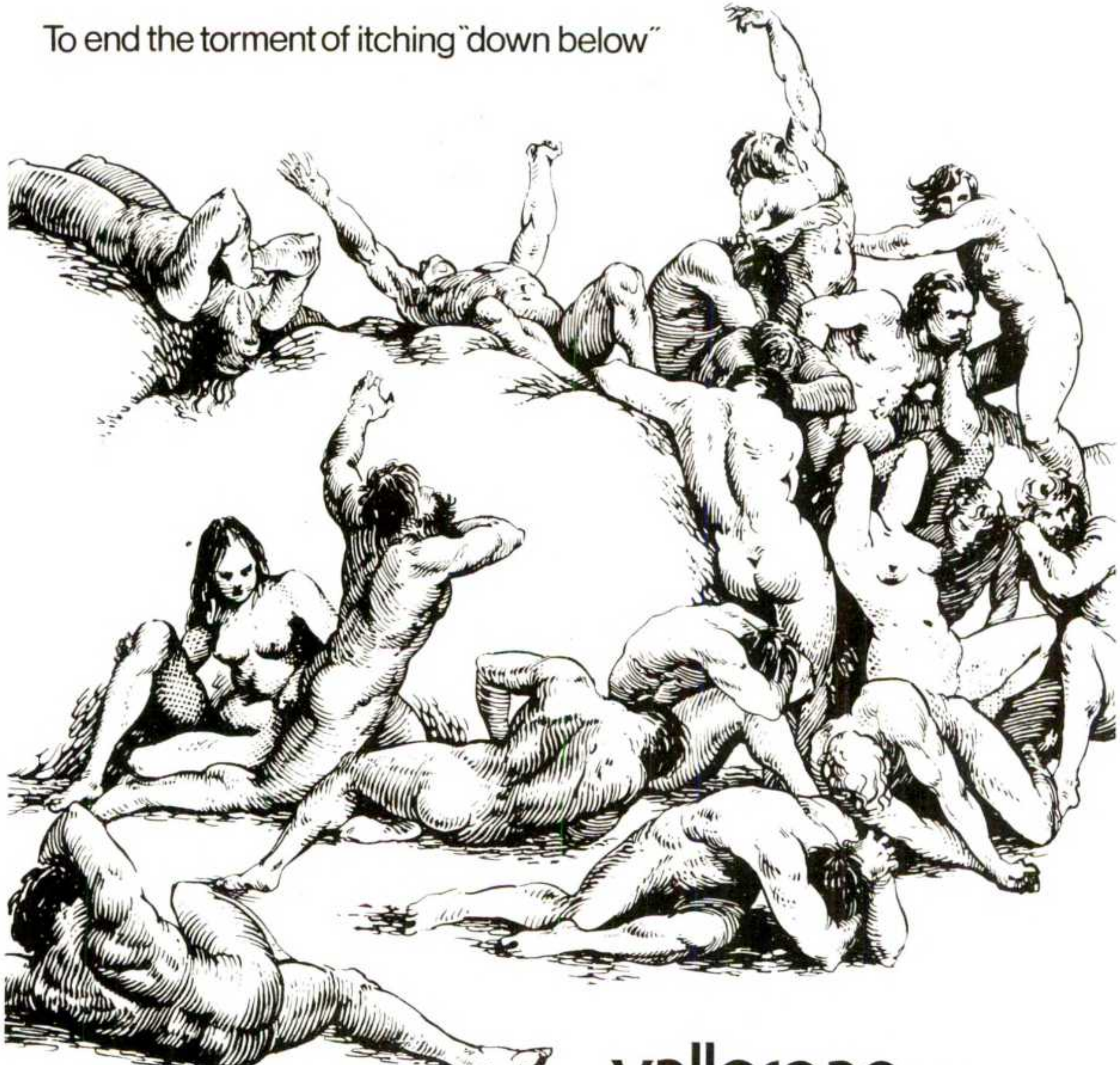
*British Medical Journal (1975) 3, 626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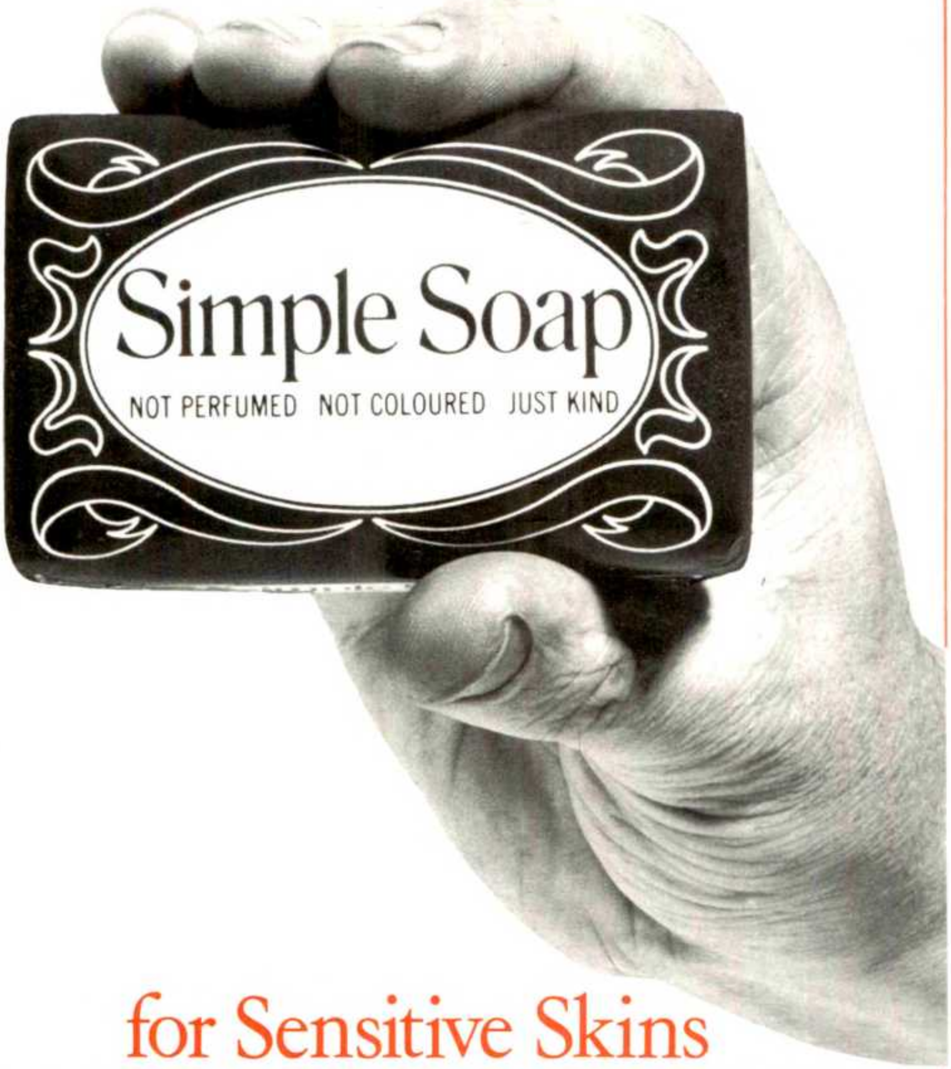

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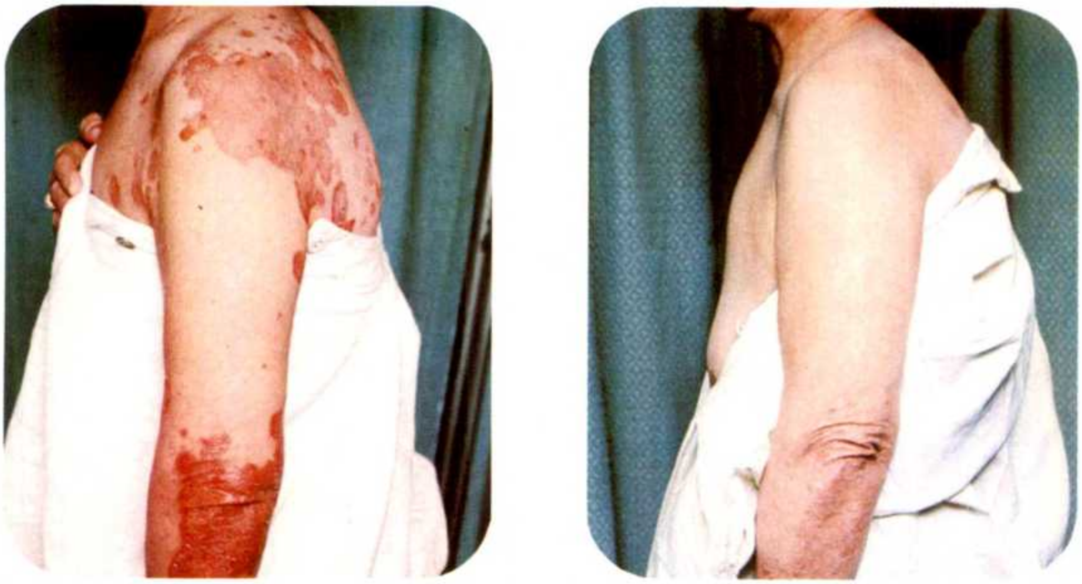

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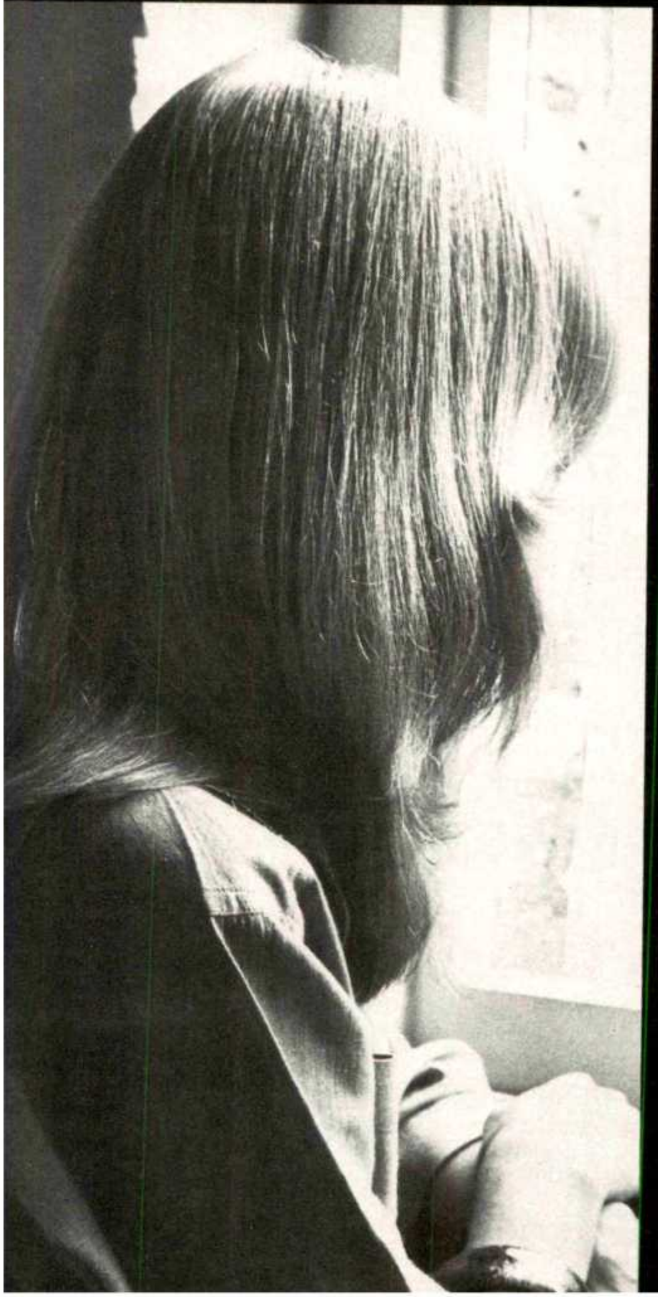

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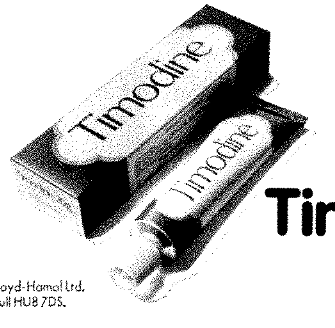

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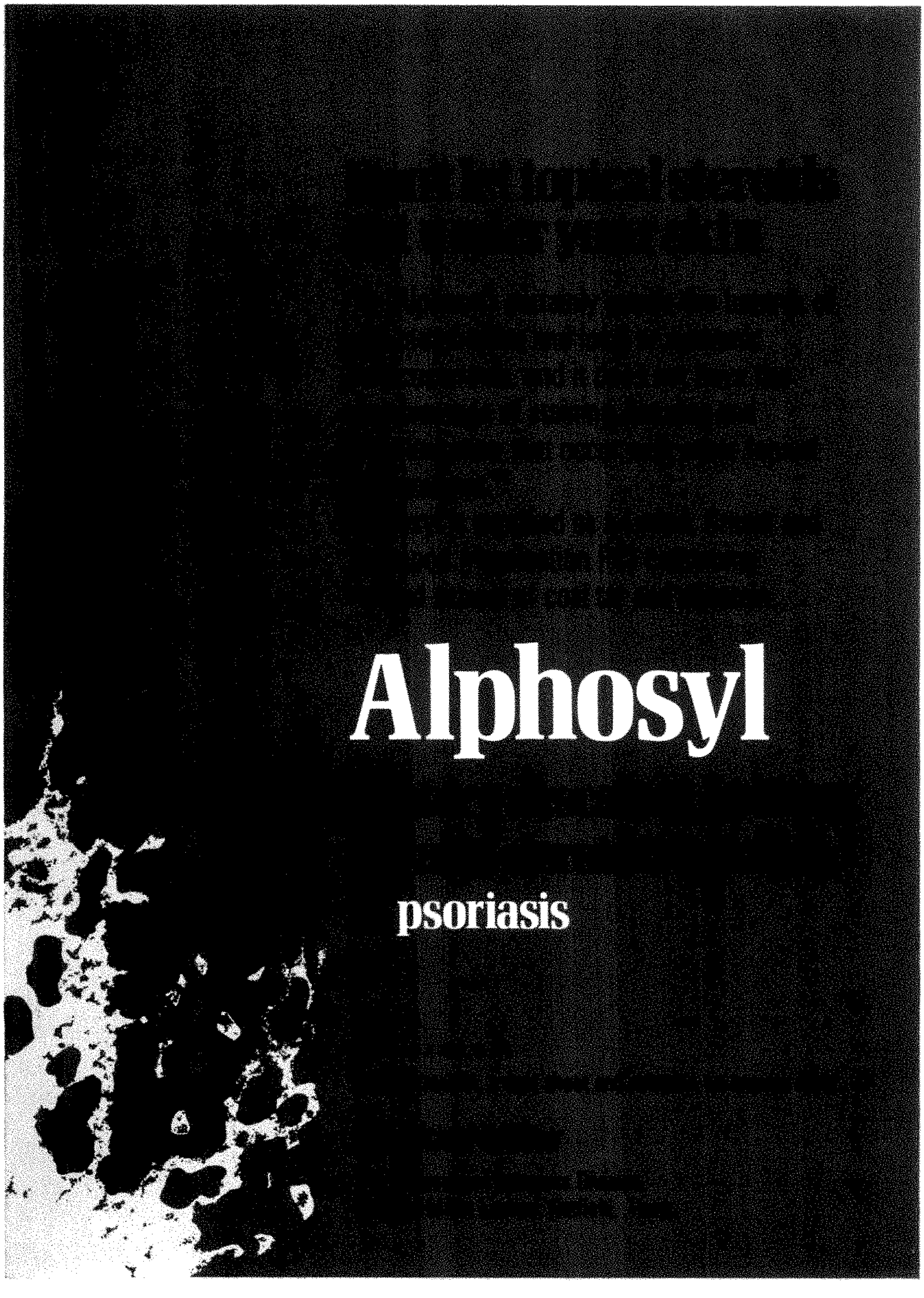

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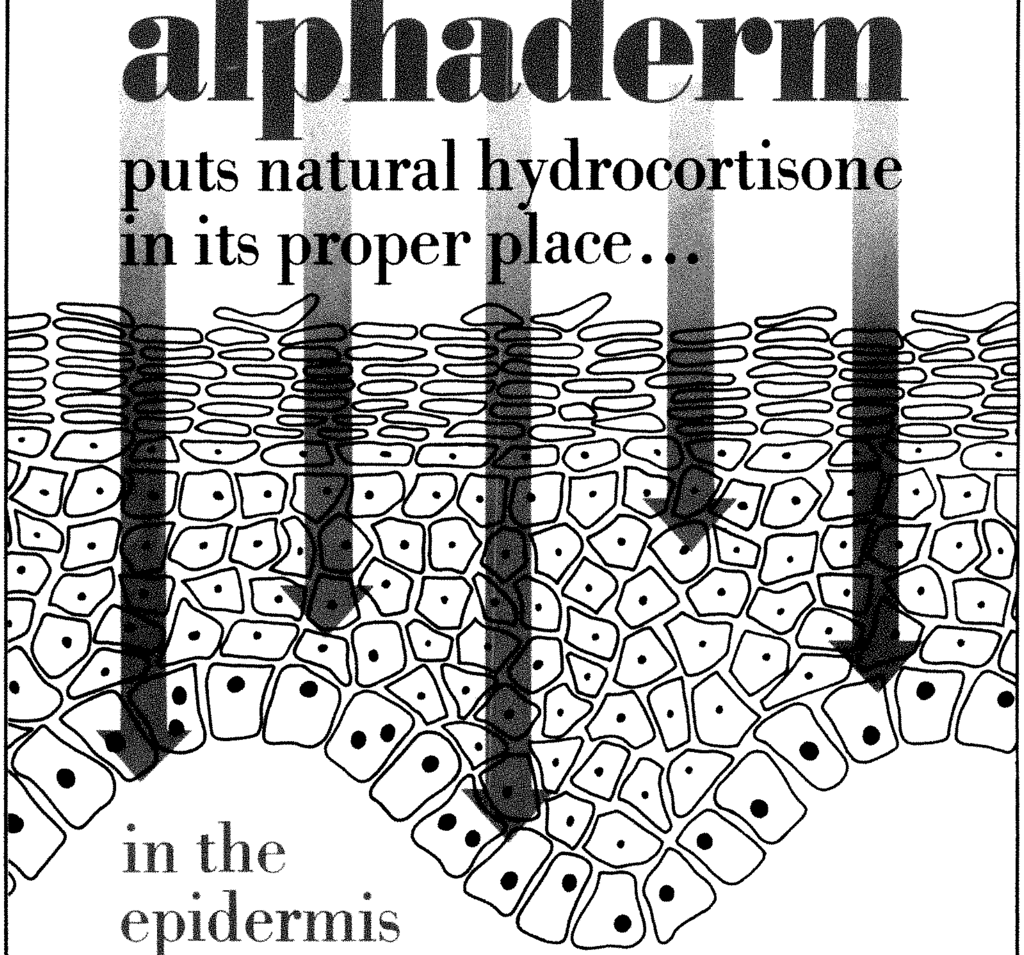

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References
1. Almeyda, J. and Burt, B.W. (1974) *Br. J. Dermatol.* **91,** 579
2. Jacoby, R.H. and Gilkes, J.J.H. (1974) *Curr.med.Res.Opin.* **2,** 474

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British Journal of Dermatology (1976) **94**, 233.

Clinical and Laboratory Investigations

Contact dermatitis from antioxidants

HIDDEN SENSITIZERS IN TOPICAL MEDICATIONS AND FOODS

JYTTE ROED-PETERSEN AND NIELS HJORTH

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SUMMARY

In a search for contact sensitivity to antioxidants we patch tested consecutive patients referred with eczematous dermatitis.

Six cases of allergic contact sensitivity to nordihydroguaiaretic acid (NDGA) were observed. Three had been sensitized by one brand of cream containing 0·1% NDGA, in three patients the source of sensitization could not be traced.

In four patients we found positive patch tests to butylated hydroxyanisole and/or to butylated hydroxytoluene. In two cases the positive patch tests were relevant, since both patients remained asymptomatic when antioxidants were avoided in food. They both had acute flares of vesicular eczema on the fingers after oral administration of small amounts.

Gallate esters and Vitamin E (d,l-alpha-tocopherol) each gave one unexplained positive patch test.

The present data suggest that further search for hidden sensitizers in topical medicaments and cosmetics is warranted. A declaration of all ingredients in industrial products should be placed on the label.

Antioxidants are added to foods, cosmetics and pharmaceuticals to prevent the oxidation of unsaturated fatty acids to small molecular weight compounds, which have an unpleasant off-flavour and a rancid taste.

Enzymatically catalysed oxidations will not be prevented by antioxidants. Auto-oxidation catalysed by metal salts (e.g. iron and copper) and ultraviolet light is reduced by incorporation of antioxidants into the lipids. This auto-oxidation is believed to occur when oxygen is added to an unsaturated chain by a free radical mechanism resulting in formation of peroxides. The antioxidant breaks this reaction by hydrogen donation and reaction with the peroxides (Johnson, 1971).

With a few exceptions the lipid-soluble antioxidants are phenols. Those favoured by industry are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), nordihydroguaiaretic acid (NDGA), esters of gallic acid (propyl-, octyl- and dodecylgallate) and tocopherols (Johnson, 1971).

An antioxidant for lipids must be fat soluble, must not impart any foreign colour, taste or flavour and must be effective even after heating and long storage. Finally it should obviously have a low acute toxicity and the daily doses ingested over the entire lifespan be without any appreciable harmful effect.

For topical use of a preservative the sensitizing properties should be analysed carefully.

In most countries such testing is not required before marketing a new compound. But even if it were, several years might elapse before the first cases of contact dermatitis were noticed. For example, although Irgasan DP 300 has been widely used as an antiseptic in toilet products for at least 8 years, contact dermatitis from it has only recently been reported (Roed-Petersen, Auken & Hjorth, 1975). In the same way reports of contact dermatitis from antioxidants are few.

MATERIALS AND METHODS

Consecutive patients referred for eczematous dermatitis of different types were patch tested with the following antioxidants: d,l-alpha-tocopherol, norhydroguaiaretic acid (NDGA), butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Three patients with one or more positive patch tests to these were further tested with propyl- and dodecylgallate. Two further cases both sensitive to NDGA were identified from the files of the department. Patch test concentrations are shown in Table 2.

TABLE 1. Phenolic antioxidants in foods and/or topical products, previously reported cases of contact sensitivity

	BHA	BHT	NDGA	Dodecylgallate	Progalin*	Tocopherols
Permitted in foods	+	+	—	+	+	+
Permitted in topical applications	+	+	+	+	+	+
No. of cases of contact sensitivity previously reported	2	0	2	5	5	9

* Progalin: dodecyl- + octyl- + propylgallate.

TABLE 2. Results of patch tests with antioxidants

Case no.	BHA 2% pet.	BHT 2% pet.	NDGA 2% pet.	Propylgallate 2% olive oil	Dodecylgallate 2% olive oil	d,l-alpha-tocopherol 20% pet.
1	+	—	+	—	+	+
2	nt	nt	+	nt	nt	nt
3	—	—	+	nt	nt	—
4	—	—	+	nt	nt	nt
5	—	+	—	—	—	—
6	+	+	+	—	—	nt
7	+	+	+	nt	nt	—
Positive	3	3	6	0	1	1
Total tested	112	112	111	(no controls)	435 1% pet. other controls (Fregert & Hjorth, 1969) 6·6% pos.	116

nt, not tested.

RESULTS

Our results are shown in Table 2. Table 1 summarizes previously reported cases of contact sensitivity from these antioxidants.

Each of the above-mentioned antioxidants is further discussed below, including previously reported investigations and our own case reports.

Gallate esters

From Switzerland ten cases of occupational contact dermatitis from gallate esters in margarine have been reported. Burckhardt & Fierz (1964) described five patients sensitized by a margarine containing 2% Progalin (dodecyl- octyl- and propylgallate). Brun (1964, 1970) described five further patients sensitized by dodecylgallate. The concentration of gallate in the margarine was not stated.

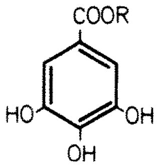

FIGURE 1. Gallate esters. R: —CH_2 CH_2 CH_3 propylgallate; —CH_2 $(CH_2)_6$ CH_3 octylgallate; —CH_2 $(CH_2)_{10}$ CH_3 dodecylgallate.

In guinea-pig experiments the sensitizing properties of propylgallate equalled those of dinitrochlorobenzene (DNCB) (Kahn, Phanuphak & Claman, 1974). Feeding propylgallate to guinea-pigs induced a specific unresponsiveness (Kahn, Phanuphak & Claman, 1974). A similar mechanism might explain why clinical contact dermatitis to gallates is rare, although man is easily sensitized.

Topical products rarely contain more than 0·02% and food 0·005–0·01% which may be too low either to induce contact sensitization or to overcome a previously established tolerance.

Tocopherols

Among the eight naturally occurring tocopherols vitamin E or alpha-tocopherol has the greatest biological activity. It occurs in plants and can be synthesized industrially. In spite of extensive use in food and topical applications few cases of contact sensitivity have been reported.

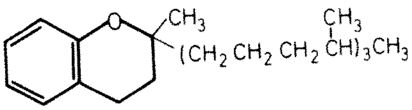

FIGURE 2. Alpha-tocopherol.

In 1965 Brodkin & Bleiberg reported a case of contact dermatitis from d-alpha-tocopherol 0·5% in a cream. In 1973 vitamin E was used in axillary deodorants and caused six reported cases of contact dermatitis, three of which were patch tested with positive results (Aeling, Panagotacos & Andreozzi, 1973; Minkin, Cohen & Frank, 1973). Many more probably occurred since the product was withdrawn from the market. Maibach & Marzulli (1974) in the analysis of such a sensitivity found the results shown in Table 3.

At this department 116 consecutive patients in 1974–75 were patch tested with d, l-alpha-tocopherol 20% in petrolatum (pet). Among these, one case of patch test sensitization was observed (Roed-Petersen & Hjorth, 1975). One patient described below (case 1) had a positive patch test on day 2 (Table 2). Another thirty-five consecutive patients patch tested with d,l-alpha-tocopherol 10% pet. had no reactions.

TABLE 3. Analysis of contact sensitivity to vitamin E by Maibach & Marzulli (1974)

	Pt. no. 1	Pt. no. 2
d, l-alpha-tocopheryl acetate 2% acetone	++	+
d-alpha-tocopherol 2% acetone	−	−
d, l-alpha-tocopherol (black) 2% alc.	−	+
d, l-alpha-tocopherol 2% in Mennen E	−	+
Natural vitamin E cream 12%	++	−
Vitamin E oil, mixed tocopherols 32% in vegetable oil	+++	−

Nordihydroguaiaretic acid (NDGA)

NDGA is used in topical products only. Previously many countries permitted its use in foods too. Only two cases of contact dermatitis from NDGA have previously been reported (Jørgensen & Hjorth, 1970). Cosmea Lanolin Cream containing 0·1% NDGA sensitized the first patient. Preparation of vitamin AD tablets at a pharmaceutical factory caused an occupational dermatitis in the second patient. Both had positive patch tests to NDGA 2% pet. The second patient had a positive patch test to oil of soybean containing 0·15% NDGA. Among 435 consecutive controls previously tested one had a weak reaction to NDGA 2% pet. while the remainder were negative (Jørgensen & Hjorth, 1970).

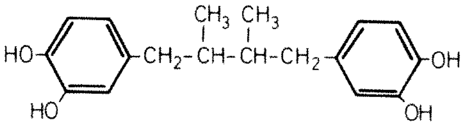

FIGURE 3. Nordihydroguaiaretic acid (NDGA).

Since 1971 we have seen six cases of contact sensitivity to NDGA. Three of the patients were sensitized by the above-mentioned lanolin cream containing 0·1% NDGA. (The composition has now been changed.)

Case reports

Case 1. A physician's wife aged 37 years had had severe ichthyosis since birth and atopic dermatitis until the age of 14. At the age of 30 she developed hand eczema which subsided 2 years before she was seen.

Upon return from a skiing holiday in April 1972, she developed overnight an acute erythroderma. Large areas of skin were oozing and suggestive of an incipient toxic epidermal necrolysis. Her hands were red and had vesicles. The eruption subsided after 2 weeks' treatment with prednisone 60 mg per day.

Bouts of itching during the holiday and the final erythroderma had been preceded by the intake of one brand of orange soda. Standard patch tests and tests with this brand were negative. Since the eruption was assumed to be a toxicoderma caused by the orange soda, further testing was abandoned.

She returned in March 1973 with an acute dermatitis on the dorsa of her hands (Fig. 4) and on the neck. The day before she had tried on her ski outfit. Patch tests with the fur and the lining of the skiing gloves were negative, and so were tests with the three fabrics of her anorak.

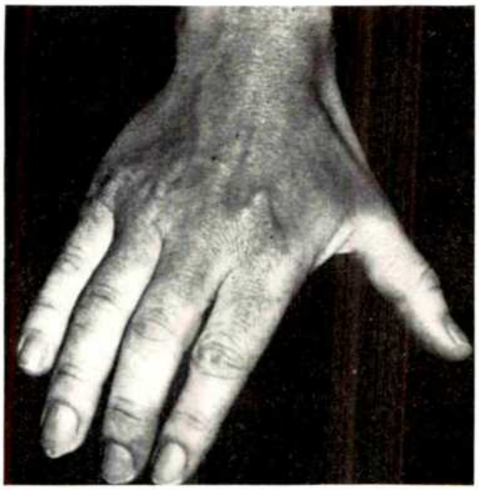

FIGURE 4. Case 1: dermatitis from NDGA.

The cause of her violent dermatitis proved to be NDGA (Table 2). While on a skiing holiday in 1972 she used Cosmea Lanoline Cream for her severe ichthyosis. This cream contained NDGA 0·1%. The acute erythroderma developed during a dinner before which she had had a bath followed by the use of Cosmea Lanoline Cream all over her body. During the holiday the ski outfit had been contaminated by the cream to such an extent that remnants in the lining of the gloves provoked a recurrence.

She remained well and after 2 years returned for further patch testing. (Table 2). She stated that about 1 year ago she had used an axillary deodorant containing vitamin E for a short period with no ill effects.

Case 2. A male lawyer aged 52 years, developed perianal eczema after 6 weeks' use of Cosmea Lanolin Cream. Patch tests showed: NDGA 2% pet. + +, Lanolin cream + +, wool alcohol 30% pet. neg. (Table 2).

Case 3. A female secretary aged 18 years developed eczema of the face 4 years previously during treatment of impetigo with Lucosil cream, containing sulphamethizole and preserved with paraben esters. She was then treated with Betnovate cream, and 4 years later she was referred with perioral dermatitis, which improved after oral tetracycline and withdrawal of the steroid cream. A few months later an acute eczema of the face developed after use of a face rinse preserved with parabens. Patch tests: benzyl-, butyl-, propyl-, ethyl- and methylparaben each 5% pet. were all + +, NDGA 2% pet. + +, BHA 2% pet., BHT 2% pet., d,l-alpha-tocopherol 20% pet. and sulphamethizole 5% pet. were negative (Table 2). The source of sensitization to NDGA could not be traced.

Case 4. A male veterinary surgeon aged 44 years had atopic dermatitis and had had asthma until the age of 15. He was referred with hand eczema of 2 years duration. Patch tests: NDGA 2% pet. + +, Carbamix 3% pet. + +, Thiurammix 1% pet. + +, spiramycin 1% pet. + +. BHA 2% pet. and BHT 2% pet were negative (Table 2). Cosmea Lanoline Cream was found to be the cause of sensitization to NDGA.

The remaining two cases with positive patch tests to NDGA are described below (cases 6 and 7). Control patch tests with NDGA 2% pet. were negative in 105 consecutive patients (Table 2).

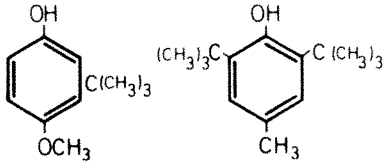

FIGURE 5. Butylated hydroxyanisole (BHA)—left; and butylated hydroxytoluene (BHT)—right.

Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT)

Meneghini, Rantuccio & Lomuto (1971), in order to determine the incidence of allergic contact sensitivity in a population of patients suffering from eczematous dermatitis of different types, performed patch tests with numerous constituents of topical medicaments, including additives. Among 360 consecutive patients they found one positive reaction to BHA 5% pet. and no reactions to BHT 5% pet. Clinical data were not included.

Degreff & Verhoeve (1975) observed one case of contact sensitivity to BHA, probably caused by the use of an antimycotic cream containing 0·005% BHA. Patch tests were positive to BHA 5% pet. and to the active ingredient in the cream (miconazole nitrate 2% pet.).

In an investigation of the depigmenting properties of BHT, Bentley-Phillips & Bayles (1974) after a usage test patch tested 50 volunteers with BHT 0·5% and 3% in a cream vehicle. Six showed a negative reaction to BHT 0·5% on day 2 but a slight irritation on day 9, and nine had a positive reaction to BHT 3% on day 9. The authors assume these reactions to be non-specific and not indicative of allergic sensitization.

Maibach, Gellin & Ring (1975) performed a similar experiment, applying BHT under occlusive tape in concentrations ranging from 0·2% to 3·3% in a cream base. Daily application for 60 consecutive days caused no sensitization in sixteen volunteers. Irritation was observed at some of the test sites and the control sites throughout.

In 1971 and 1974–75 we patch tested a total of 112 patients with BHA 2% pet. and BHT 2% pet. Two patients had positive reactions to both, one to BHA only and one to BHT only (Table 2). Eighty-three consecutive patients tested with BHA 5% and BHT 5% in alcohol were all negative.

Case 5. A 24-year-old female had had eczema of the hands for 6 months, and of the feet and lips for 3 months. Various steroid creams did not control the eruption. Standard patch tests, and scratch tests with proteins were negative. Patch tests with chicory salad and allylisothiocyanate 0·1% pet. were positive. BHT 2% pet. + +; BHA 2% pet., NDGA 2% pet., propyl- and dodecylgallate 2% in olive oil (o.o) and d,l-alpha-tocopherol 20% pet. were negative (Table 2).

A diet composed of foods without antioxidants, and continued local treatment with a steroid cream cleared the eczema in 2 weeks.

The patient then took a tablet containing BHA 10 mg and BHT 10 mg. Twelve hours later she noticed itching followed by a vesicular eczema localized to several fingers and the vermilion border of her lower lip. This eruption disappeared after 2 weeks' continued diet and local treatment.

We suspected the patch test positive BHT to have caused the eruption and repeated the provocation by giving BHA 5 mg daily orally for 4 days. She then had to stop because of a new eruption of acute eczema of her hands, which once again cleared under diet and local treatment. Further provocation tests with BHT were abandoned because she became pregnant.

When the patient was almost free of symptoms 5% alcoholic solutions of BHA and BHT were swabbed, each on one finger. After 15 min both of the treated areas itched, and shortly after an urticarial reaction developed. The symptoms subsided in about 1 hour.

Case 6. A 74-year-old nickel sensitive lady had had a mild hand eczema of 12 years duration and kept under control by use of steroid creams. The eczema suddenly worsened. Patch tests showed: nickel sulphate 5% pet., dimethyl-butyl-phenyl-PPD 1% pet., wool alcohols 30% pet., eucerin 20% pet., NDGA 2% pet., BHT 2% pet. and BHA 2% pet. were all + +. Propylgallate 2% o.o, dodecylgallate 2% o.o and d,l-alpha-tocopherol 5% pet. were negative (Table 2).

The eczema cleared under continued local treatment with a steroid cream and a diet free of antioxidants. The importance of the additional diet appeared from the following provocation test:

Oral administration of BHA 10 mg per day for 4 days resulted in a vesicular itching eruption on the second finger of the left hand, where the eczema originally was most pronounced. The eruption cleared in 1–2 weeks under continued diet and local treatment. Since then she has been asymptomatic. The source of sensitization by antioxidants could not be traced.

Biopsy of a positive patch test (BHA 2% pet.) confirmed that the reaction was allergic and not irritant (Dr K.-E. Sjølin).

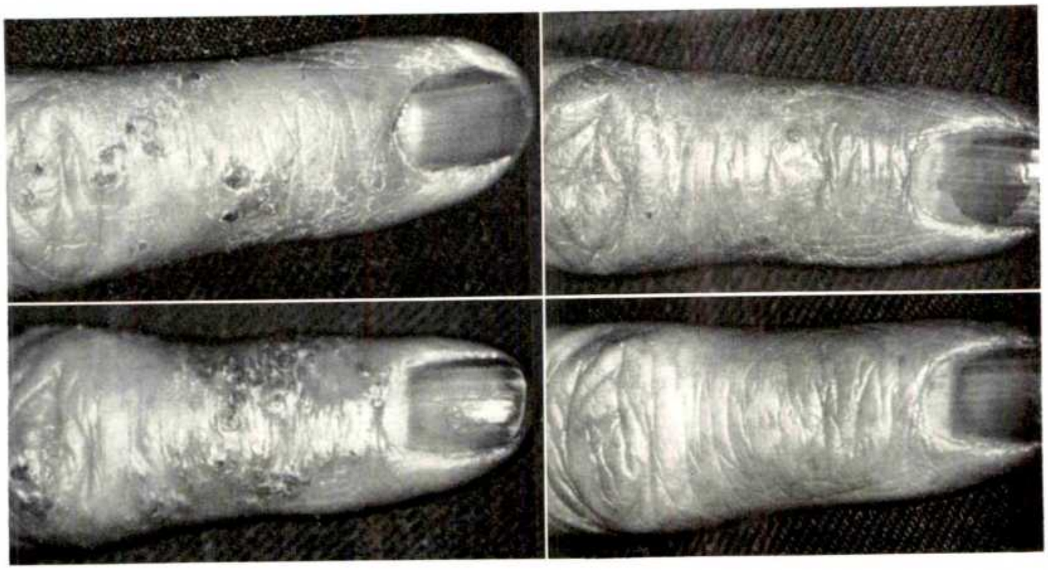

FIGURE 6. Case 6: female aged 72 years, positive patch tests to BHA and BHT.
Top left: 3 February 1975, before diet free of antioxidants.
Top right: 24 February 1975, on diet free of antioxidants.
Bottom left: 7 March 1975, eruption of vesicles after oral provocation test with BHA 10 mg daily for 4 days.
Bottom right: 21 March 1975, again on diet free of antioxidants.

Case 7. A 67-year-old female had a leg ulcer of 35 years duration. Some years before she twice had eczema after use of Lucosil ointment, containing sulphamethizole and preserved with paraben esters.

In 1974 she had a period with irritation and redness of her oral mucosa. These symptoms subsided after withdrawal of toothpaste. Patch tests were positive to constituents of toothpaste.

Patch test results: carvone 5% pet., spearmint 5% pet., eugenol 2% pet., benzyl cinnamate 2% pet., styrax 2% pet., gum benzoin 2% pet., wood tars, balsam of Peru 25% pet., tolubalsam 10% alc., amercol L lol, p-phenylenediamine 1% pet., dicyclohexyl-PPD 1% pet., dimethyl-benzyl-phenyl-PPD 1% pet., mercury 0·5% pet., NDGA 2% pet., BHA 2% pet. and BHT 2% pet. were all + + (Table 2).

She had probably been sensitized by topical remedies used for treatment of the leg ulcer. However, although we found so many positive patch tests she had no dermatitis.

COMMENTS

In the concentration used the sensitizing potential of antioxidants must be low. Three of six persons sensitive to NDGA in this series and one of the two previous cases had with great certainty been sensitized by one brand of cream, containing the unusually high concentration of 0·1% NDGA. Five of the previously described cases of occupational dermatitis from gallate esters were sensitized by a margarine containing 2% of these antioxidants. The maximum permitted in Denmark is at present 0·01% of gallate esters in food. When allergy to preservatives in creams was first found common (Hjorth, 1961) this group of hidden sensitizers was not usually declared on the labels of pharmaceutical topical agents. Today dermatologists expect to be informed of such preservatives. The present paper indicates that further search for hidden sensitizers in creams is now warranted. Antioxidants should be declared on the list of ingredients given by the manufacturers of topical medicaments.

Sensitivity to antioxidants may explain the sensitization in some cases, which have given otherwise negative results. It is known that patch tests with a cream base containing parabens 0·3% will give negative results in many patients later found to have a relevant reaction to parabens 15% in petrolatum (Hjorth, 1961). Likewise sensitivity to antioxidants will not necessarily be demonstrable by a positive reaction to the cream actually used. It is worth while stressing that the search for antioxidant sensitivity described in this paper was based on demonstration of sensitivity to NDGA in a cream for which the contents were declared. Sensitivity to BHA and BHT was found as an incidental result of their inclusion in a standard patch test series. Biopsy and control patch tests confirmed that the reactions were allergic and not irritant. The frequency of antioxidant sensitivity is unknown.

As with paraben sensitivity the frequency is too low to be of interest to the general population. We hope that this paper will not cause a totally unfounded scare of antioxidants as occurred with parabens. These were abandoned by some pharmaceutical firms, who find it an effective advertising point to stress that parabens have been substituted by other preservatives. Some antioxidants have an unknown sensitizing potential, while others are possibly much more allergenic than the parabens.

The same conclusions were stated by Marzulli & Maibach (1974). In a review of the status of topical parabens used during 50 years they concluded that the sensitization rate is low, and that alternatives to the parabens ought to be carefully studied, so that they do not surprise us and prove to be a greater topical or systemic hazard.

It is well known that many epidermal allergens cause various types of skin reactions when they are ingested by sensitive individuals. Recently Christensen & Möller (1975) showed that ingestion of nickel impurities in the daily food intake was a major cause of chronicity in nickel sensitive females with hand eczema.

British Journal of Dermatology (1976) 94, 243.

Incorporation of 1-^{14}C-acetate into the lipids of isolated epidermal cells

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Accepted for publication 7 July 1975

SUMMARY

Suspensions of epidermal cells were prepared by trypsinization of rat epidermis and incubated with 1-^{14}C-acetate in Eagle's minimum essential medium. The incorporation of radioactivity into the total lipids of the cells was increased by the addition of serum to the medium. The pattern of incorporation into the main lipid classes was affected by the addition of serum to the medium and by alterations in the density of the cell suspensions. The incorporation of radioactivity into the total lipids increased approximately linearly over a 24-h period of incubation and the pattern of incorporation into the phospholipids and glycolipids altered with time. However, at each time interval studied, reproducible patterns of incorporation were obtained under controlled conditions. The incorporation of radioactive acetate therefore provides the basis for a very sensitive method of lipid analysis.

There have been numerous studies on the surface lipids of skin, but comparatively few attempts to measure the relative contributions of the epidermis and sebaceous glands to synthesis of skin lipids. In a study of lipogenesis, with various fractions of cultured human skin, Hsia *et al.* (1970) showed that the sebaceous glands synthesized predominately squalene and triglycerides whereas the epidermis synthesized relatively greater proportions of sterols and polar lipids. Similar results were obtained by Summerly & Woodbury (1971) who cultured human skin in the presence of ^{14}C-acetate and then separated the intact sebaceous glands from the overlying appendage-freed epidermis by treatment with calcium chloride. However, it is possible to examine the epidermal contribution more directly by studying lipogenesis in isolated epidermal cells. Wilkinson (1970) has shown that isolated epidermal cells readily incorporate ^{14}C-acetate into fatty acids and Prottey, Hartop & Ferguson (1972) have shown that similar patterns of incorporation into the lipids can be obtained with isolated cells and with intact epidermis.

We are primarily interested in the phospholipids and glycolipids as these are essential structural components of the membrane systems of the cell and may be involved in the process of keratinization. The measurement of the radioactivity incorporated into the various lipid classes not only demonstrates the nature of the lipids synthesized by the epidermal cell but also provides the basis for a very sensitive method of lipid analysis. However, the practical application of the method is entirely dependent on the ability to reproduce consistently the same patterns of labelling in different preparations of cells under a given set of experimental conditions. An attempt to develop such a method is described in this paper.

MATERIALS AND METHODS

Animals

In the preliminary experiments germ-free rats were used, but for the later experiments the skin pelts were shaved and then cleaned with a 5% solution of cetrimide. The rats were killed by exposure to chloroform.

Isolation of cells

All the apparatus and solutions used in the following procedure were sterilized.

The skin pelt was stretched over a dampened sponge and shaved with electric clippers. The pelt was thoroughly cleaned with a 5% solution of cetrimide followed by several washes with water. Swabs were taken from several areas of the skin pelt and cultured on nutrient agar plates and in thioglycollate broth, to check for sterility.

Strips of skin (0·4 mm thick) were cut with a Castroviejo keratotome (John Weiss & Son, London) and floated (dermis side down) on 0·1% solution of trypsin (Sigma type III) in phosphate buffered saline (Dulbecco A, Oxoid Ltd.) containing benzyl penicillin (100 u/ml), streptomycin sulphate (100 u/ml) and Fungizone (amphotericin B, Squibb; 25 u/ml). The strips of skin were incubated for 1 h at 37°C. The epidermis was separated from the dermis with forceps and shaken twice with 2×10 ml of a solution of horse serum (Wellcome No.3; 7·5 ml) in phosphate buffered saline (18·7 ml). The whole suspension was filtered through a nylon gauze (132 μm hole size; J.Staniar & Co., Manchester) to give a preparation of epidermal cells in a volume of 20 ml. The cell suspension was centrifuged (800 *g*) at 4°C for 15 min, the supernatant removed and the cells re-suspended in a solution of phosphate buffered saline. The sterility of the cell suspension was checked by plating out on nutrient agar plates and thioglycollate broth. Cell counts were performed in a Neubauer counting chamber (depth 0·1 mm) and cell viability was assessed by exclusion of the dyes, nigrosin and eosin.

Incubation of cells with $1\text{-}^{14}C$*-acetate*

The cells were suspended in an appropriate volume of Eagle's minimum essential medium (MEM) with or without the addition of 10% calf serum, dispersed by gentle pipetting and incubated at 37°C under an atmosphere of carbon dioxide/air (5:95) in Petri dishes (3·5 cm diameter) or in Falcon flasks. Sodium $1\text{-}^{14}C$-acetate (62 mCi/mmol; Radiochemical Centre, Amersham) was dissolved in phosphate buffered saline and added to the incubation medium to give a final concentration of 10 μCi/ml. After incubation, the sterility of each incubation mixture was checked as previously described.

Extraction of lipids from cells

The cell suspensions were centrifuged at 800 *g*, the medium was removed and the cells were washed twice by re-suspension in phosphate buffered saline. The lipids were extracted by shaking the cells with chloroform–methanol (2:1 v/v) overnight. After centrifuging to remove the cell debris, the lipid extracts were taken to dryness three times with nitrogen to dissociate proteo-lipids. The residues were re-dissolved in chloroform–methanol (2:1 v/v), washed by the method of Folch, Lees & Sloane-Stanley (1957), and dried over anhydrous sodium sulphate.

Fractionation of lipids

(a) *Column chromatography.* Total lipids were separated by chromatography on columns of silica gel H (0·5 g) into (a) neutral lipids, (b) acidic phospholipids and glycolipids, (c) choline-containing phospholipids by the method of Gray (1967).

(b) *Thin-layer chromatography of phospholipids and glycolipids.* Pre-coated thin layer plates (20 × 20 cm) of silica gel 60 (Merck A.G., Darmstadt, Germany) were used without activation.

The acidic phospholipids and glycolipids, together with reference compounds, were applied to the plates in chloroform–methanol (2:1 v/v) and under an atmosphere of nitrogen. The plates were developed in the first dimension with chloroform–methanol–water (65:35:4 by vol.) and in the second dimension with tetrahydrofuran–methylal–methanol–4N ammonia (10:4:6:1 by vol.).

The choline-containing phospholipids, together with reference compounds, were applied to thin layer plates of silica gel in chloroform–methanol (2:1 v/v) and under an atmosphere of nitrogen. The plates were developed in chloroform–methanol–water (65:35:4 by vol.).

The following reference compounds were used:

Phosphatidyl inositol, phosphatidyl serine, phosphatidyl ethanolamine, cardiolipin, phosphatidyl glycerol, cholesterol sulphate, ceramide, ceramide monohexoside, ceramide dihexoside, phosphatidyl choline, sphingomyelin and lysophosphatidyl choline.

Identification of the radioactive compounds was made by co-chromatography with the above reference compounds which were visualized by spraying the plates with 40% sulphuric acid and heating at 160°C or by staining with iodine vapour. The phospholipids were deacylated with mild alkali (Kates, 1972) whilst the sphingolipids and glycosphingolipids were not hydrolysed under these conditions.

Measurement of radioactivity

The thin layer plates were exposed to X-ray film (Kodirex) for 5–14 days. The areas of silica gel containing the radioactive compounds were located from the autoradiographs, scraped into scintillation vials, and 10 ml of a solution of 2-(4-tert-butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole (butyl-PBD, BDH) in toluene was added to each vial. The radioactivities were measured by a Nuclear Chicago liquid scintillation spectrometer with an external standard to determine the counting efficiencies.

Preparation of methyl esters of fatty acids from the triglycerides

The neutral lipids and reference compounds were applied under nitrogen to a thin layer (0·5 mm) plate (20 × 20 cm) of silica gel G (Merck A.G., Darmstadt, Germany) which had been activated at 110°C. for 30 min. The plate was developed with light petroleum (boiling point range: 30–40°C)–diethyl ether–glacial acetic acid (80:20:1 by vol.), dried under nitrogen, sprayed with an aqueous solution of rhodamine 6G (0·01%) and the compounds visualized under ultra-violet light. The area of silica gel containing the triglycerides was scraped immediately from the plate and extracted three times with chloroform–methanol (2:1 v/v; 3 ml). The combined extracts were taken to dryness under nitrogen, dissolved in methanol–sulphuric acid (95:5 v/v; 3 ml), gassed with nitrogen and heated at 70°C for 2 h. The methyl esters of the fatty acids were extracted and analysed on a Pye 'series 104' instrument equipped with a flame ionization detector and a 2 m column of 10% polyethylene glycol adipate at a temperature of 184°C (Long, 1972). The radioactivities of the fatty acid methyl esters were measured by the method of Yardley & Long (1972).

RESULTS

Incorporation of 1-^{14}C-acetate into lipids of epidermal cells

(a) *The effect of serum in the medium.* In all the experiments there was a greater incorporation of

TABLE 1. Incorporation of 1-^{14}C acetate into total lipids of epidermal cells

Experiment no.	Cell density (10^6/ml)	Radioactivity (c/100s)* MEM+ serum	Radioactivity (c/100s)* MEM	Ratio of uptake MEM+serum/MEM
1	12·4	535,852	198,132	2·7
2	10·7	579,238	246,606	2·3
3	7·9	343,698	40,936	8·4
4	8·9	315,015	40,014	7·9

Time of incubation, 24 h.
* Mean of duplicates.

TABLE 2. Pattern of incorporation of 1-^{14}C acetate into main lipid classes

Lipid class	% of total radioactivity MEM*	% of total radioactivity MEM+serum*
Squalene, sterol/wax ester	4·9	2·3
Triglyceride	28·9	20·9
Fatty acid	8·0	2·4
Unknown	9·3	7·0
Sterol/diglyceride	12·7	19·2
Phospholipid/glycolipid monoglyceride	36·2	48·2

Time of incubation, 24 h.
* Mean of 4 experiments in duplicate.

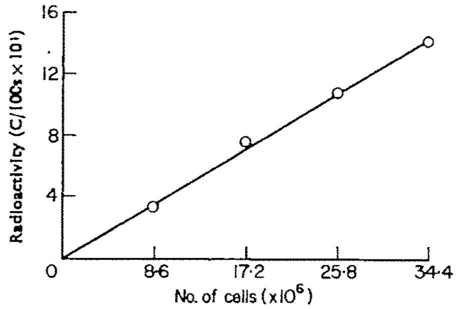

FIGURE 1. Incorporation of 1-^{14}C-acetate into the total lipids of epidermal cells.

In two of our cases, 'dyshidrotic' eczema of the hands could be provoked by oral exposure to the antioxidant, while healing took place when the patients avoided these substances in their diet. Both of these ladies preferred the hardship of avoiding antioxidants rather than to suffer from eczema, but it must be realized what a social handicap such a restriction imposes on the individual.

REFERENCES

Aeling, J.L., Panagotacos, P.J. & Andreozzi, R.J. (1973) Contact dermatitis to vitamin E aerosol deodorant. *Archives of Dermatology*, **108**, 579.

Bentley-Phillips, B. & Bayles, M.A.H. (1974) Butylated hydroxytoluene as a skin lightener. *Archives of Dermatology*, **109**, 216.

Brodkin, R.H. & Bleiberg, J. (1965) Sensitivity to topically applied vitamin E. *Archives of Dermatology*, **92**, 76.

Brun, R. (1964) Kontaktekzem auf laurylgallat und p-hydroxy-benzoesaureester. *Berufsdermatosen*, **12**, 281.

Brun, R. (1970) Eczema de contact a un antioxydant de la margarine (gallate) et changement de metier. *Dermatologica*, **140**, 390.

Burckhardt, von W. & Fierz, U. (1964) Antioxydanten in der Margarine als Ursache von Gewerbeekzemen. *Dermatologica*, **129**, 431.

Christensen, O.B. & Möller, H. (1975) External and internal exposure to the antigen in the hand eczema of nickel allergy. *Contact Dermatitis* (in press).

Degreff, H. & Verhoeve, L. (1975) Contact dermatitis to miconazole nitrate. *Contact Dermatitis* (in press).

Fregert, S. & Hjorth, N. (1969) Results of standard patch tests with substances abandoned. *Contact Dermatitis Newsletter*, **5**, 85.

Hjorth, N. (1961) *Eczematous allergy to balsams*, pp. 97–99. Munksgård, Copenhagen.

Johnson, F.C. (1971) *A critical review of the safety of phenolic antioxidants in foods* (Ed. T.E. Furia). Chemical Rubber Co., Cleveland, Ohio.

Jørgensen, G. & Hjorth, N. (1970) Dermatitis from nordihydroguaiaretic acid, an antioxidant in fats. *Contact Dermatitis Newsletter*, **7**, 151.

Kahn, G., Phanuphak, P. & Claman, H.N. (1974) Propyl gallate contact sensitization and orally induced tolerance. *Archives of Dermatology*, **109**, 506.

Magnusson, B. & Kligman, A.M. (1970) *Allergic contact dermatitis in the guinea-pig*. pp. 102–120. Charles C. Thomas, Springfield, Illinois.

Maibach, H. & Marzulli, F. (1974) personnal communication.

Maibach, H., Gellin, G. & Ring, M. (1975) Is the antioxidant butylated hydroxytoluene a depigmenting agent in man? *Contact Dermatitis* (in press).

Marzulli, F. & Maibach, H. (1974) Status of topical parabens: skin hypersensitivity. *International Journal of Dermatology*, **13**, 397.

Meneghini, C.L., Rantuccio, F. & Lomuto, M. (1971) Additives, vehicles and active drugs of topical medicaments as causes of delayed-type allergic dermatitis. *Dermatologica*, **143**, 137.

Merck Index 1968. Merck & Co. Inc. Rahway, N.J. USA.

Minkin, W., Cohen, H.J. & Frank, S.B. (1973) Contact dermatitis from deodorants. *Archives of Dermatology*, **107**, 774.

Roed-Petersen, J., Auken, G. & Hjorth, N. (1975) Contact dermatitis from Irgasan DP 300. *Contact Dermatitis* (in press).

Roed-Petersen, J. & Hjorth, N. (1975) Patch tests sensitization from d,l-alpha-tocopherol. *Contact Dermatitis* (in press).

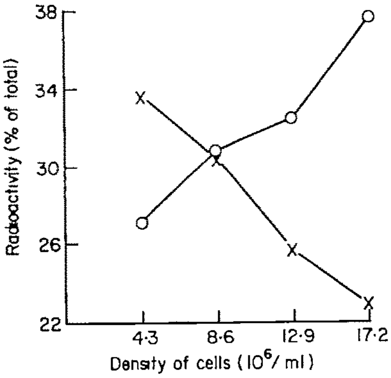

FIGURE 2. Change in the pattern of incorporation of 1-^{14}C-acetate into the lipids of epidermal cells with alteration in cell density. O——O, triglycerides; ×——×, phospholipids.

radioactivity into the total lipids when serum was present in the culture medium (final concentration 10%). The addition of serum to the medium produced a 2–8 fold increase in the total incorporation (Table 1).

The presence of serum in the medium also affected the pattern of incorporation of radioactivity into the main lipid classes. There was proportionately less incorporation into the triglycerides and more incorporation into the phospholipids and the sterols and diglycerides (Table 2).

TABLE 3. Fatty acid composition of triglycerides from epidermal cells

Peak no.	Identity	% of total*	
		Mass	Radioactivity
1	14:0	1·5	7·6
2		1·3	1·3
3		0·4	0·0
4	16:0	25·0	46·3
5	16:1	4·8	2·5
6		2·2	1·8
7		1·0	0·2
8	18:0	10·5	6·8
9	18:1	26·2	3·4
10	18:2	18·9	12·3
11	18:3	0·7	0·9
12	20:0	0·7	4·3
13		0·4	2·7
14		1·2	4·0
15		4·8	3·8
16	22:0	0·4	2·1
	Saturated	38·1	67·1
	Unsaturated	50·6	19·1
	Unknown	11·3	13·8

* Mean of duplicate determinations.

TABLE 4. Incorporation of 1-^{14}C-acetate into total lipids of epidermal cells

No. of cells ($\times 10^6$)	Cell density ($\times 10^6$/ml)	Radioactivity (c/100s)* Time of incubation 19 h	Radioactivity (c/100s)* Time of incubation 24 h
20	2·0	1,021,520	1,214,640
20	2·0	514,550	584,750
21	2·1	713,310	865,250

* Mean of duplicates.

TABLE 5. Pattern of incorporation of 1-^{14}C-acetate into the phospholipids and glycolipids of epidermal cells

Lipid	% of total radioactivity Experiment 25 19 h	Experiment 25 24 h	Experiment 26 19 h	Experiment 26 24 h
Phosphatidyl serine	3·0	1·3	2·7	2·1
Phosphatidyl inositol	2·5	1·8	1·6	2·2
Cardiolipin	0·9	0·8	0·6	0·5
Phosphatidyl ethanolamine	3·7	3·1	4·8	4·0
Phosphatidyl glycerol	2·8	2·3	2·8	2·0
Ceramide monohexoside	31·9	36·5	33·3	34·4
Fast glycolipid	2·5	2·5	4·0	4·1
Phosphatidyl choline	33·7	33·9	40·1	39·1
Sphingomyelin	4·4	5·2	5·4	7·1
Lysophosphatidyl choline	2·4	1·6	0·6	0·9
Unidentified compounds	12·2	11·0	4·1	3·6
Total acidic phospholipids and glycolipids	58·1	59·0	53·4	52·6
Total choline phospholipids	41·9	41·0	46·6	47·4

TABLE 6. Incorporation of 1-^{14}C-acetate into total lipids of epidermal cells

No. of cells ($\times 10^6$)	Cell density ($\times 10^6$/ml)	Radioactivity (c/100s)* Time of Incubation 4 h	Radioactivity (c/100s)* Time of Incubation 21 h
20	2·0	330,945	1,232,590
21	2·1	363,310	1,282,440
21	2·1	184,640	1,099,550

* Mean of duplicates.

TABLE 7. Pattern of incorporation of 1-^{14}C-acetate into the phospholipids and glycolipids of the epidermal cells

Lipid	% of total radioactivity*			
	Experiment 28		Experiment 29	
	4 h	21 h	4 h	21 h
Phosphatidyl serine	3·0	2·8	2·2	2·5
Phosphatidyl inositol	6·0	2·0	6·2	2·0
Cardiolipin	2·2	1·9	2·0	1·2
Phosphatidyl ethanolamine	8·4	6·0	6·7	4·5
Phosphatidyl glycerol	3·5	1·8	2·7	1·5
Ceramide monohexoside	6·1	32·5	5·2	37·6
Fast glycolipid	3·3	3·3	3·8	4·2
Phosphatidyl choline	54·7	29·2	54·0	30·0
Sphingomyelin	4·0	3·7	5·3	3·8
Lysophosphatidyl choline	1·6	1·3	5·0	1·9
Compound 10	0·5	6·4	0·5	4·5
Unidentified compounds	6·7	10·1	6·4	6·3
Total acidic phospholipids and glycolipids	38·3	64·9	33·3	63·4
Total choline phospholipids	61·7	35·1	66·7	36·6

* Mean of duplicates.

TABLE 8. Pattern of incorporation of 1-^{14}C-acetate into the phospholipids of epidermal cells

Lipid	% of total radioactivity*			
	Experiment 28		Experiment 29	
	4 h	21 h	4 h	21 h
Phosphatidyl serine	3·3	4·3	2·4	4·4
Phosphatidyl inositol	6·6	3·1	6·9	3·3
Cardiolipin	2·4	3·0	2·2	2·0
Phosphatidyl ethanolamine	9·3	9·4	7·4	7·8
Phosphatidyl glycerol	4·0	2·9	3·0	2·6
Phosphatidyl choline	60·6	45·5	61·0	52·5
Sphingomyelin	4·5	5·7	5·8	6·7
Lysophosphatidyl choline	1·8	1·9	5·5	3·3
Compound 8	0·8	3·3	0·6	2·8
Compound 10	0·6	10·0	0·6	7·8
Unidentified	6·1	10·9	4·6	8·8

* Mean of duplicates.

(b) *The effect of cell density.* The incorporation of radioactivity into the total lipids increased linearly with the number of cells (Fig. 1). However, the pattern of incorporation into the main lipid classes altered with changes in cell density. There was relatively less incorporation into the phospholipids and relatively more incorporation into the triglycerides as the cell density increased (Fig. 2). All further experiments were performed in Falcon flasks with the concentration of cells arbitrarily fixed at 2×10^6/ml.

(c) *Nature of fatty acids synthesized.* The triglycerides were very radioactive and were therefore used to provide an indication of the type of fatty acids synthesized from 1-^{14}C-acetate by the epidermal cell. Transmethylation of the triglycerides and radio-gas chromatography of the fatty acid methyl esters showed that palmitic acid was the major labelled fatty acid (Table 3).

Incorporation of radioactivity into the lipids at fixed cell densities

The epidermal cells were still incorporating radioactivity into the total lipids after 19 h of incubation (Table 4). After 19 and 24 h of incubation, ceramide monohexoside and phosphatidyl choline were the major labelled polar lipids, together accounting for about 70% of the total incorporation (Table 5). The incorporation of radioactivity into the total lipids (Table 6) increased approximately linearly with time and the pattern of incorporation in the phospholipids and glycolipids altered with the time of incubation. There was a proportionately large increase in incorporation into ceramide monohexoside accompanied by a relative decrease in incorporation into phosphatidyl choline between 4 and 21 h of incubation (Table 7). The phospholipid pattern did not alter so markedly, but there was a significant increase in incorporation into two unidentified compounds (compounds 8 and 10) after 21 h of incubation (Table 8). The proportion of viable cells in the cell suspension was 63% prior to incubation, but after 4 h of incubation the proportion of viable cells had fallen to 26% and after 21 h of incubation only 11% of the cells were still viable.

DISCUSSION

Wilkinson (1970) found that the highest incorporation of radioactivity into the total lipids of isolated epidermal cells was obtained using Eagle's minimum essential medium (MEM) and that 1-^{14}C-acetate was a better precursor than ^{14}C-glucose. With MEM he found that the incorporation of acetate was greater in the absence of serum during the first 24 h period of culture, but that if the cells were cultured for 24 h and then supplied with the precursor for a further 24 h period, the cells in medium containing serum incorporated about 10 times as much radioactivity as those deprived of serum. In all my experiments, however, there was a greater incorporation of radioactivity into the total lipids when serum was present in the medium and even during the first 24 h period of culture. Consequently, in all the subsequent experiments serum was added to the culture medium. However, the addition of serum to the medium also affected the pattern of incorporation into the main lipid classes. Prottey, Hartop & Ferguson (1972) found that the incorporation of radioactivity into the total lipids was similar in several media and that the addition of serum did not affect either the total incorporation or the pattern of labelled lipids. In contrast, Rothblat (1969) stated that serum in the medium influences both the rate and the nature of lipids synthesized. It would appear that the effect of serum on lipogenesis is complex and that the effects observed depend on the source of the cells and previous history. Thus, Bailey (1964) showed that labelled acetate incorporation into cellular phospholipids was inhibited by 86% when L cells were grown in the presence of serum and further studies by Bailey *et al.* (1972) showed that cells preferentially utilized serum lipids as a source of cellular lipid. However, all these studies were made on established cell lines which were no longer differentiated and so freshly isolated adult cells will not necessarily behave in a similar manner.

The incorporation of radioactivity into the total lipids increased linearly with the number of cells. However, the pattern of incorporation into the main lipid classes altered with changes in cell density and so all further experiments were carried out at cell concentrations arbitrarily fixed at 2×10^6/ml which is a convenient concentration for cell culture. Even under these controlled conditions, the distribution of radioactivity between the neutral and polar lipids varied from animal to animal. This may reflect the ability of the epidermal cell to store neutral lipids or it could reflect damage to the cells during preparation. However, the patterns of incorporation of 1-^{14}C-acetate into the main lipid classes of rat epidermal cells were similar to those obtained by Prottey *et al.* (1972) for rat epidermal cells and those of Summerly, Woodbury & Yardley (1972) for rat epidermis.

An indication of the type of fatty acids synthesized from 1-^{14}C-acetate by the rat epidermal cell was obtained from the pattern of triglyceride fatty acids which was similar to that of Prottey *et al.* (1972) for rat epidermis in that palmitic acid was the major labelled fatty acid. However, in contrast to Prottey *et al.* (1972), radioactivity was detected in octadecadienoic acid and in fatty acids of chain length greater than C18. These findings are in agreement with those of Wilkinson (1970) for human epidermal cells and with those of Vroman, Nemecek & Hsià (1969) for human skin. Nikkari (1965) has found fatty acids of chain length up to C26 in rat surface lipid.

The effect of time of incubation on the incorporation of radioactivity into the lipids was studied at 4, 19, 21 and 24 h. The incorporation of radioactivity into the total lipids increased approximately linearly over a 24 h period of incubation and the pattern of incorporation into the lipids altered with time. However, at each time interval, reproducible patterns of incorporation into the phospholipids and glycolipids were obtained under the controlled conditions. The technique of incorporation of radioactive acetate into the lipids can therefore provide the basis for a very sensitive method of lipid analysis.

After 4 h of incubation phosphatidyl choline accounted for approximately 50% of the total incorporation into the polar lipid, but after 21 h of incubation the pattern had changed. This was mainly due to a proportionately large increase in incorporation into ceramide monohexoside between 4 and 21 h, although the pattern of incorporation into the phospholipids also altered during this period. After 21 h of incubation, phosphatidyl choline accounted for about 30% and ceramide monohexoside for about 35% of the incorporation into the polar lipids. The changes in the phospholipid pattern and especially the accumulation of radioactivity into two unknown phospholipids (compounds 8 and compounds 10) with time may reflect the decrease in the viability of the cells that occurred during incubation. The accumulation of radioactivity in ceramide monohexoside may be caused by the absence in the epidermal cell of an enzyme capable of catabolizing this glycolipid. During the process of keratinization, the phospholipids of the epidermal cell are catabolized (Long, 1970, 1975), while the neutral lipids and glycolipids are retained by the squamous cells (Gray, 1975).

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REFERENCES

BAILEY, J.M. (1964) Lipid metabolism in cultured cells. *Proceedings of the Society for Experimental Biology and Medicine*, 115, 747.

Bailey, J.M., Howard, B.V., Dunbar, L.M. & Tillman, S.F. (1972) Control of lipid metabolism in cultured cells. *Lipids*, **7**, 125.

Folch, J., Lees, M. & Sloane-Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, **226**, 497.

Gray, G.M. (1967) Chromatography of lipids. 1. An improved chromatographic procedure for the quantitative isolation of the neutral ceramide-containing glycolipids from mammalian tissues. *Biochimica et biophysica Acta*, **144**, 511.

Gray, G.M. (1975) Unpublished results.

Hsia, S.L., Fulton, J.E., Fulghum, D. & Buch, M.M. (1970) Lipid synthesis from acetate-1-^{14}C by suction blister epidermis and other skin components. *Proceedings of the Society for Experimental Biology and Medicine*, **135**, 285.

Kates, M. (1972) Mild alkaline deacylation of phosphatides and glycolipids. In: *Techniques of Lipidology; Isolation, Analysis and Identification of Lipids* (Ed. by T.S.Work and E.Work), p. 558. North Holland, Amsterdam.

Long, V.J.W. (1970) Variations in lipid composition at different depths in the cow snout epidermis. *Journal of Investigative Dermatology*, **55**, 269.

Long, V.J.W. (1972) Changes in the fatty acid composition of the phospholipids, triglycerides, and free fatty acids with depth in the cow snout epidermis. *British Journal of Dermatology*, **87**, 227.

Long, V.J.W. (1975) Phospholipase A and lysophospholipase activity of the epidermis. *British Journal of Dermatology*, **92**, 603.

Nikkari, T. (1965) Composition and secretion of skin surface lipids of rat. Effect of dietary fat and hormones. *Scandinavian Journal of clinical and Laboratory Investigation*, **17**, Suppl. 85.

Prottey, C., Hartop, P.J. & Ferguson, T.F.M. (1972) Lipid synthesis in rat skin. *British Journal of Dermatology*, **87**, 586.

Rothblat, G.H. (1969) Lipid metabolism in tissue culture cells. *Advances in Lipid Research*, **7**, 135.

Summerly, R. & Woodbury, S. (1971) The *in vitro* incorporation of ^{14}C-acetate into the isolated sebaceous glands and appendage-freed epidermis of human skin. *British Journal of Dermatology*, **85**, 424.

Summerly, R., Woodbury, S. & Yardley, H.J. (1972) The effect of eicosa 5:8:11:14 tetraynoic acid on skin lipid synthesis (^{14}C incorporation) *in vitro*. *British Journal of Dermatology*, **87**, 608.

Vroman, H.E., Nemecek, R.A. & Hsia, S.L. (1969) Synthesis of lipids from acetate by human preputial and abdominal skin *in vitro*. *Journal of Lipid Research*, **10**, 507.

Wilkinson, D.I. (1970) Incorporation of acetate-1-^{14}C into fatty acids of isolated epidermal cells. *Journal of Investigative Dermatology*, **54**, 132.

Yardley, H.J. & Long, V.J.W. (1972) Radio–gas chromatography by collection of radioactive compounds issuing from a gas chromatograph on a moving strip of paper. *Journal of Chromatography*, **73**, 235.

British Journal of Dermatology (1976) **94**, 253.

The relationship between the course of psoriasis and transepidermal water loss, photoelectric plethysmography and reflex photometry

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SUMMARY

The transepidermal water loss (TWL), photoelectric plethysmography and reflex photometry were parallelly registered on the involved hands and forearms of psoriatics. The TWL values showed a fairly good correlation with the clinical course of the disease: they increased during the active phase and decreased after return to normal. The vascular tests did not show a strict parallelism with the clinical state. On the basis of the findings, the temporal relations between epidermal and vascular factors during the different phases of the psoriatic process are discussed. The TWL can be used to predict a relapse occurring on the area investigated but not on a distant area.

Several methods can be used to follow the healing processes in psoriasis as well as the effect of treatment. In addition to clinical observations, histological changes may be followed, especially the reformation of the granular layer (Burks & Montgomery, 1943; Goodwin, Hamilton & Fry, 1973), and there are several methods for recording the altered epidermal cell kinetics (Weinstein & Van Scott, 1965; Weinstein & Frost, 1968; Flaxman & Chopra, 1973; Steigleider, Schumann & Lennartz, 1973). All these techniques require repeated skin biopsies. Other methods are based on investigations of the altered reactivity of papillary vessels in psoriasis (Gilje, 1953; Telner & Fekete, 1961; Ross, 1964; Illig, 1966; Ryan, 1969). Persistence of abnormal capillaries after treatment with dithranol, tar and steroids has been investigated by histochemical methods (Braun-Falco, 1963; Suurmond, 1965; Braun-Falco & Christophers, 1974). In particular, the therapeutic effect of topical steroids was studied by vasoconstriction tests, i.e. the McKenzie & Stoughton test (1962) and its several modifications, as well as by recording vasoconstriction by piezoelectric and photoelectric pulse plethysmography (Thune, 1971), by reflexion photometry (Zaun & Altmayer, 1973), or by spectrophotometry (Tring, 1973). Pulse plethysmography has also been used to follow the regression of psoriatic vascular changes during dithranol or tar therapy (Thune, 1971). It was also observed that the reactivity to histamine, Trafuril and kallikrein is reduced in psoriatic skin (Holti, 1964; Michaëlsson, 1970). The significance of such vascular tests was the demonstration of functional changes in skin vessels in the macroscopically intact, 'healed' psoriatic skin (Lawler & Vineyard, 1960; Ross, 1964). We have investigated the state of the vessels by photoelectric plethysmography and by reflectometry as well as measuring an epidermal parameter, the transepidermal water loss (TWL). TWL is known to be increased in psoriasis presumably due to an altered corneal barrier function in that disease (Felsher & Rothman, 1945; Grice & Bettley, 1967). Both dermal (vascular) and epidermal parameters have been studied because of the old observations that both these skin layers have a role in the mechanism of psoriasis, a fact recently stressed by Braun-Falco & Christophers (1974).

METHODS AND RESULTS

Patient material. Clinically and histologically verified psoriatics of both sexes were investigated and were given the routine Ingram and Goeckerman regime.

Reflectometric test

The colour of the eruption (see below) was recorded by a photoelectric reflection meter (Photovolt 670). In short-time measurements the filling of the subpapillary venous plexus is the most important factor influencing the colour. An increased reflection of the photometer is produced by constriction of the subpapillary plexus, whereas a red colour indicates dilatation of these vessels. Other contributory factors influencing the reflection are the colours of the surface layers of the epidermis and the amount and distribution of skin pigments, especially melanin. Typical plaques on the backs of the hands or on the volar or dorsal aspects of the forearm were selected and the same areas measured at weekly intervals.

The results are shown in Fig. 1, in which it is seen that fourteen of twenty-three cases showed improved values during therapy.

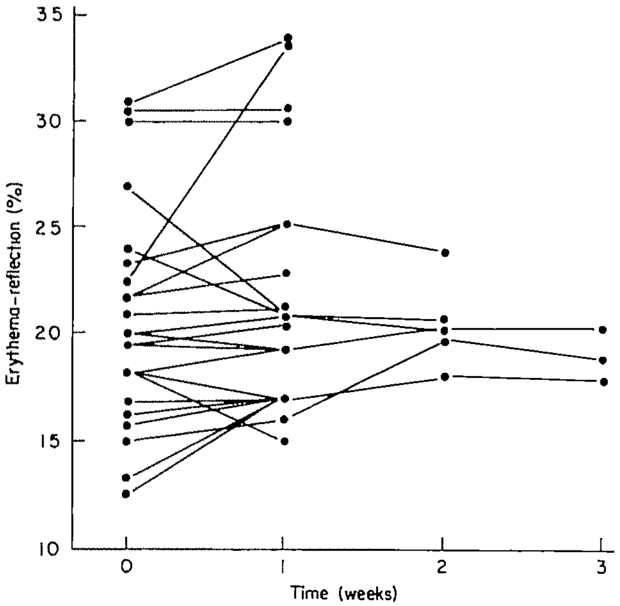

FIGURE 1. Reflex erythema measurements in twenty-three cases of psoriasis treated by the Ingram or Goeckerman regime.

TWL tests

The TWL was measured by a Meeco electrolytic water analyser. Nitrogen (water content less than 0·5 p.p.m.) was led through a teflon tube at a constant flow of 100 ml/h into a cup with a diameter of 0·5 cm which was fixed onto the skin; the water content of the nitrogen led from the cup was then measured in the apparatus. The values were recorded by a Metrohm Labograph E 478 for ½ h. Only values after 5 min were considered, as a constant level was generally reached by this time. The values given in p.p.m. were then calculated in mgH_2O/cm^2. This technique corresponds to the method of van Gasselt & Vierhout (1963), as modified by Thiele & Malten (1972). The patients were sitting in a comfortable position and the aim of the study was explained to them to reduce possible emotional reactions leading to sweating. The skin temperature was estimated on the backs of the hands or forearms by an electric 'Universal' thermometer (Ellab Type TE 3). The measurements were performed under almost identical conditions of room temperature (22–24°C) and relative humidity (33–37%) and at the same time of the day in winter and spring.

Investigations were performed:

(a) in eighteen cases on typical plaques on the backs of the hands and the forearms at weekly intervals.

(b) in forty-four cases on macroscopically unaffected skin of psoriatics on the backs of the hands or forearms. Similar studies were performed on the normal skin of fifteen patients with eczematous lesions and in sixteen patients without skin diseases, at weekly intervals.

The results for (a) are given in Fig. 2 which shows an improvement in the values at the end of the observation period in fourteen out of the eighteen psoriatic patients. In several cases prior deterioration of the TWL was registered. By further analysis it was found that on extremely thick plaques the TWL values were low and with continued therapy became first higher and then tended to decrease towards normal values.

The results for (b) show that there were no significant differences between the TWL values in psoriatics and in controls in macroscopically unaffected skin.

Parallelly performed plethysmographic, reflectometric and TWL tests

The method of photoelectric pulse plethysmography has been described previously (Hertzman, Randall & Jochim, 1945; Nieven, van der Slikke & Reichert, 1965; Thune, 1970; Thune & Rajka, 1974). The sensor was equipped with cadmium sulphide as photosensitive material and was gently fixed to the skin during investigation. The pulse curve was recorded on an oscilloscope and was photographed directly. All readings were obtained at a room temperature between 21 and 25°C. An increase in pulse indicates arteriolar dilatation and *vice versa*. The other skin tests were performed as described above. Involved skin on the backs of the hands or forearms was tested at weekly intervals during a hospital stay of at least 3 weeks in fifteen psoriatic in-patients and initial values were compared with values at discharge from hospital.

The results are shown in Table 1. It is evident that parallel results occurred in seven of the fifteen tested cases. Of these fifteen cases, improvement was observed by TWL estimation in ten, by reflex erythema measurement in eleven and by plethysmography in eleven. The skin temperature fell in general and these values showed a certain but not strict parallelism with the TWL and plethysmographic values.

Comparison between tests and clinical conditions

In order to evaluate which of these three parameters fits best with the clinical conditions, nine extremely therapy-resistant cases were tested. In patients 1 and 2 the same tests were also performed

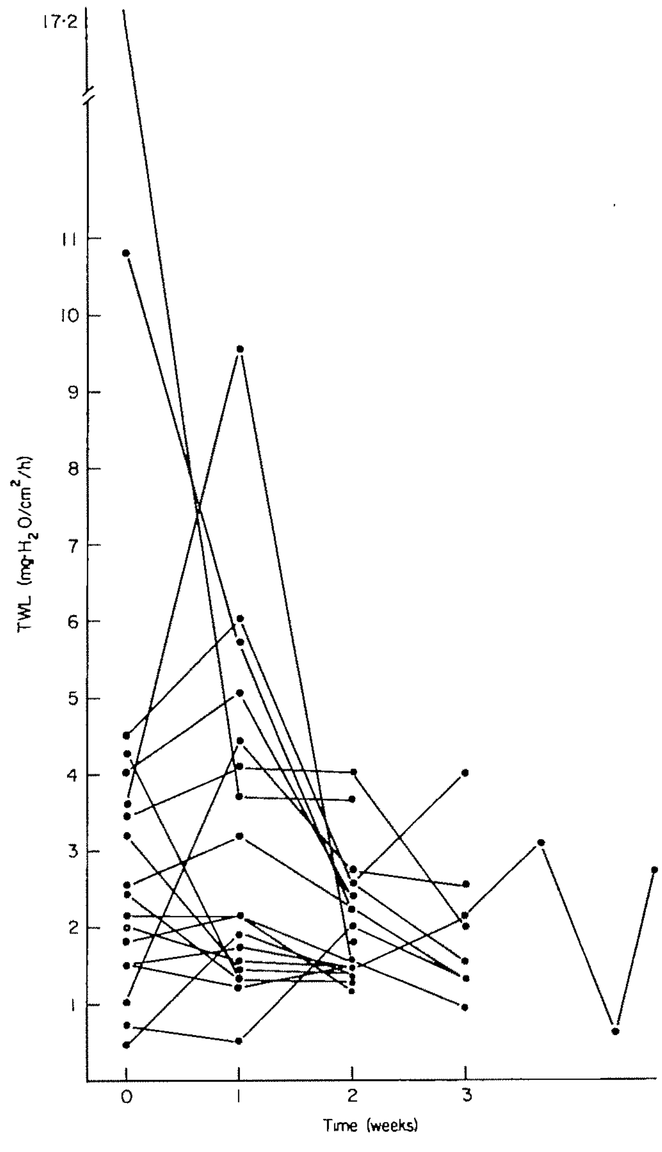

FIGURE 2. Transepidermal water loss changes in eighteen cases of psoriasis treated by the Ingram or Goeckerman regime.

during a second period of hospitalization which followed soon after the first period. The clinical appearance of the tested plaques was recorded.

The results are shown in Fig. 3 which demonstrates that improved test results were less often seen in these nine cases than in the foregoing studies. Especially at the end of the observation period a reversal of the initially improved values was often seen.

The clinical course showed a strict parallel with the TWL values whereas several discrepancies were recorded between the vascular test and the clinical state.

TABLE 1. Parallel tests during 3–6 weeks antipsoriatic therapy (Ingram or Goeckerman regime)

Patients	TWL	Reflectometry	Photoelectric plethysmography
1	Reduced	Increased	Reduced
2	Reduced	Increased	Reduced
3	Reduced	Increased	Reduced
4	Reduced	Increased	Reduced
5	Reduced	Increased	Reduced
6	Reduced	Increased	Reduced
7	Reduced	Increased	Reduced
8	Unchanged	Increased	Reduced
9	Increased	Increased	Reduced
10	Increased	Reduced	Reduced
11	Reduced	Unchanged	Reduced
12	Reduced	Unchanged	Reduced
13	Reduced	Reduced	Unchanged
14	Unchanged	Increased	Reduced
15	Increased	Increased	Reduced

Temporal relations between tests and clinical condition

During several months the vascular parameters and the TWL were followed at weekly intervals in every psoriatic in the in-patient department and at the same time the clinical state was recorded. The patients were given routine treatment, as mentioned above, and clinical deterioration was especially looked for. Fourteen such cases could be evaluated with special reference to any alteration in the tested parameters appearing before the clinical deterioration.

Prior deterioration in the tests was observed: in five cases in TWL and plethysmographic values; in three cases in TWL and reflectometric values; in four cases in only TWL values; in one case in only reflectometric value. Thus the TWL values deteriorated in twelve of fourteen cases before the clinical exacerbation. In most cases these changes were parallel with the vascular tests which showed early changes in only six (plethysmographic), and four (reflectometric) of the fourteen cases.

TWL tests in patients after hospital discharge

According to Fig. 3, patients 1 and 2 showed initial clinical improvement due to local therapy in hospital, and then relapsed soon after discharge despite continued local treatment with topical steroids and tar preparations. Generally the TWL values showed a good parallel with the clinical state and we therefore followed up these values in eleven cases after discharge. Patients leaving the hospital were re-examined for TWL values at 3-weekly intervals and their clinical condition was recorded.

The TWL values are collected in Fig. 4 which shows improvement in two cases, a deterioration in three cases, while three cases were biphasic and three unchanged. The clinical condition showed a fairly good correlation in all cases. However, this was only valid for the plaque tested. Thus in some cases, although the tested plaque was clinically improved or had even disappeared and the corresponding TWL values were significantly improved, new lesions could be seen in other areas as a sign of clinical relapse.

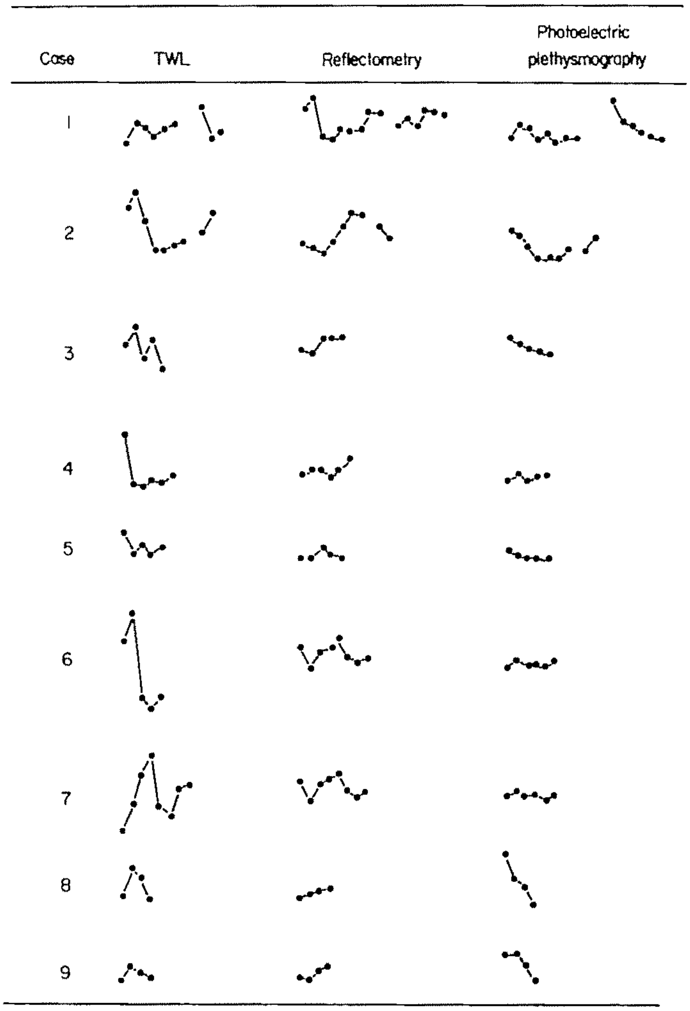

FIGURE 3. Transepidermal water loss, reflectometric and photoelectric plethysmographic values in nine cases of psoriasis resistant to local therapy.

DISCUSSION

In connection with the reflectometric studies, the colour changes recorded over longer periods of time could also have been influenced by the presence of skin pigment. Increased melanin formation is in general characteristic during the later phases of the psoriatic process, and a close relationship between pigmentation and microvascular changes is assumed (Ryan, 1970). Also dithranol and tar therapy may contribute to increased pigmentation.

Furthermore the reflectometric technique as well as the photoelectric plethysmography method cannot be considered as sufficiently sensitive methods to demonstrate minor changes in the subpapillary venular or arteriolar vessels.

By means of the Meeco apparatus the TWL values can be measured on smaller skin areas than are used for the vascular tests. This has to be considered when comparing the results of TWL and of the

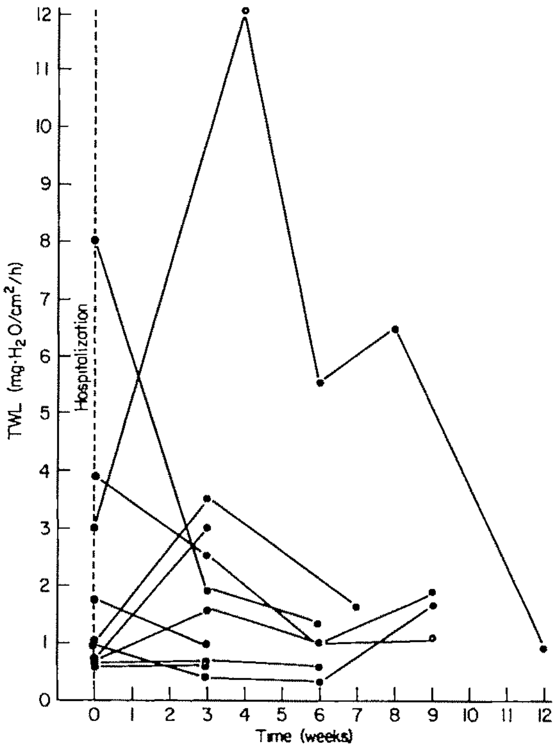

FIGURE 4. Transepidermal water loss values in psoriatics after discharge from the department.

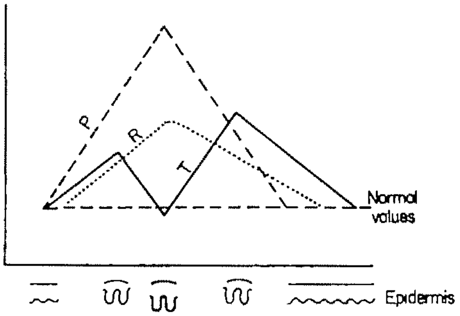

FIGURE 5. Changes in transepidermal water loss, plethysmographic and reflectometric values during the course of the psoriatic process. P, plethysmography; R, reflectometry; T, TWL.

vascular tests. To avoid non-physiological conditions, the TWL values have been measured without blocking sweat secretion by anticholinergic drugs (total evaporative skin water loss was measured, i.e. TWL and sweat).

When we measured early lesions which later developed into thick plaques, the TWL increased in the initial phase. The TWL was very low through some thick plaques. When after continued treatment these plaques lost their scales and decreased in thickness, the TWL also increased and then with continued treatment gradually decreased (Fig. 5).

A factor which may result in a lowering of the TWL is the sweat duct obstruction in psoriasis

(Sulzberger, Herrmann & Zak, 1947; Halter, 1950; Cormia & Kuykendall, 1955; Juhlin, 1967; Johnson & Shuster, 1969). Halter observed that anhidrosis may precede the psoriatic eruption. Juhlin (1967), counting the increase of functioning sweat pores after different local treatments in psoriatics, saw least improvement after steroids (fluocinolone). But he concluded that this method could not be used to predict relapses.

Shadidullah *et al.* (1969), using an electrolytic hygrometer, also found a gradual reduction in TWL in the involved skin of patients with eczematous conditions, and in some cases the 'normal appearance was regained a few days before TWL returned to normal'. In our present studies the TWL change has preceded the clinical deterioration of psoriasis. When considering the healing of the psoriatic process (see also Table 1), the impression is that there is a tendency to show an earlier return to normal (in particular in plethysmography) in the vascular tests, than in the TWL values. On the other hand, it is not possible to evaluate the effects of the different treatments (tar, dithranol, UVR) on the epidermal, and dermal processes. In addition, it cannot be excluded that some patients may have also used local corticosteroids for short periods. It was practically impossible to avoid topical steroid treatment during the long observation time in our patients. Mostly for technical reasons no definite conclusion could be drawn from the present studies on the time course of the psoriatic process. By our methods it seemed that the epidermal changes appeared before and became normal later than the vascular changes did. The arteriolar reduction shown by plethysmography began earlier but also became normal earlier than the subpapillary venous changes tested by reflectometry (Fig. 5).

The question arises whether the measurement of the TWL values can be used as a practical test to predict when a patient will relapse. In the present studies this seemed to be the case if an individual lesion was frequently monitored, but the method could not predict a relapse distant from the plaque investigated. Therefore it is advisable to study several plaques in the areas where such tests may practically be performed. Even so, a prediction of relapse cannot be given with certainty. The measurement of TWL (on several plaques) may nevertheless be a useful method with a reasonable degree of reliability to predict a clinical relapse.

REFERENCES

Braun-Falco, O. (1963) Morphogenese der psoriatischen Hautreaktion. *Archiv für Klinische und Experimentelle Dermatologie*, **216**, 130.

Braun-Falco, O. & Christophers, E. (1974) Structural aspects of initial psoriatic lesions. *Archiv für Dermatologische Forschung*, **251**, 85.

Burks, J.W. & Montgomery, H. (1943) Histopathologic study of psoriasis. *Archives of Dermatology and Syphilology*, **48**, 479.

Cormia, F.E. & Kuykendall, V. (1955) Studies in sweat retention in various dermatoses. *Archives of Dermatology*, **71**, 425.

Felsher, Z. & Rothman, S. (1945) Insensible perspiration of skin in hyperkeratotic conditions. *Journal of Investigative Dermatology*, **6**, 271.

Flaxman, B.A. & Chopra, D.P. (1973) Cell cycle of normal and psoriatic epidermis *in vitro*. *Journal of Investigative Dermatology*, **59**, 102.

Gasselt, Van, H.R.M. & Vierhout, R.R. (1963) Registration of the insensible perspiration of small quantities of sweat. *Dermatologica*, **127**, 255.

Gilje, O. (1953) Capillary microscopy in the differential diagnosis of skin diseases. *Acta dermato-venereologica (Stockholm)*, **33**, 103.

Goodwin, P.G., Hamilton, S. & Fry, L.A. (1973) A comparison of the effect of various topically active steroids on the clinical and histological features of psoriasis. *British Journal of Dermatology*, **89**, 61.

Grice, K.E. & Bettley, F.R. (1967) Skin water loss and accidental hypothermia in psoriasis, ichthyosis and erythroderma. *British medical Journal*, **iv**, 195.

HALTER, K. (1950) Zur Prüfung vegetativer Funktionen bei Dermatosen. *Archiv für Dermatologie und Syphilis (Berlin)*, **191**, 134.

HERTZMAN, A.B., RANDALL, W.S. & JOCHIM, K.E. (1945) The estimation of cutaneous blood flow with the photoelectric plethysmograph. *American Journal of Physiology*, **145**, 716.

HOLTI, G. (1964) Vascular phenomena of Dermatology. *British Journal of Dermatology*, **76**, 503.

ILLIG, L. (1966) Die Blutgefäss-Reaktion bei der Psoriasis vulgaris. Histologische und capillarmikroskopische Untersuchungen. *Archiv für Klinische und Experimentelle Dermatologie*, **255**, 424.

JOHNSON, C. & SHUSTER, S. (1969) Eccrine sweating in psoriasis. *British Journal of Dermatology*, **81**, 119.

JUHLIN, L. (1967) Effect of treatment on the vascular compartment of the psoriatic lesion. *Archives of Dermatology*, **82**, 190.

LAWLER, J.C. & VINEYARD, W.R. (1960) The effect of treatment on the compartment of the psoriatic lesion. *Archives of Dermatology*, **86**, 190.

MCKENZIE, A.W. & STOUGHTON, R.B. (1962) Method for comparing percutaneous absorption of steroids. *Archives of Dermatology*, **86**, 608.

MICHAËLSSON, G. (1970) Decreased cutaneous reactions to kallikrein in patients with atopic dermatitis and psoriasis. *Acta dermato-venereologica (Stockholm)*, **50**, 37.

NIEVEN, J.L., VAN DER SLIKKE, L.B. & REICHERT, W.J. (1965) Photoelectric plethysmography using reflected light. *Cardiologica, (Basel)*, **29**, 160.

ROSS, J.B. (1964) The psoriatic capillary. Its nature and value in the identification of the unaffected psoriatic. *British Journal of Dermatology*, **76**, 511.

RYAN, T.J. (1969) A study of the epidermal capillary unit in psoriasis. *Dermatologica*, **138**, 459.

RYAN, T.J. (1970) Factors influencing the growth of vascular endothelium in the skin. *British Journal of Dermatology, Suppl.* **5**, **82**, 99.

SHADIDULLAH, M., RAFFLE, E.J., RIMMER, A.R. & FRAIN-BELL, W. (1969) Transepidermal water loss in patients with dermatitis. *British Journal of Dermatology*, **81**, 722.

STEIGLEDER, G.K., SCHUMANN, H. & LENNARTZ, K.J. (1973) Autoradiographic *in vitro* examination of psoriasis skin before, during and after dithranol treatment. *Archiv für Dermatologische Forschung*, **246**, 231.

SULZBERGER, M.B., HERRMANN, F. & ZAK, F.G. (1947) Studies in sweating. I. Preliminary report with particular emphasis on a sweat retention syndrome. *Journal of Investigative Dermatology*, **23**, 345.

SUURMOND, D. (1965) Histologic changes in treated and untreated psoriatic lesions. *Dermatologica*, **131**, 357.

TELNER, P. & FEKETE, Z. (1961) The capillary responses in psoriatic skin. *Journal of Investigative Dermatology*, **36**, 225.

THIELE, F.A.J. & MALTEN, K.E. (1972) Insensible water loss. *Transactions of the St John's Hospital Dermatological Society*, **58**, 199.

THUNE, P. (1970) Plethysmographic recordings of skin pulses. II. Piezoelectric and photoelectric measurements in psoriasis. *Acta dermato-venereologica (Stockholm)*, **50**, 263.

THUNE, P. (1971) Plethysmographic recordings of skin pulses. III. Effect of corticosteroids, dithranol and tar in psoriasis. *Acta dermato-venereologica (Stockholm)*, **51**, 183.

THUNE, P. & RAJKA, G. (1974) Investigations of thurfyl nicotinate and methacholine reactions in atopic dermatitis by photoelectric reflectometry and plethysmography. *Archiv für Dermatologische Forschung*, **250**, 258.

TRING, F.C. (1973) Skin colour changes in psoriasis responding to treatment with betamethasone-17-valerate under occlusion. *Acta dermato-venereologica (Stockholm)*, **54**, 199.

WEINSTEIN, C.D. & VAN SCOTT, E.J. (1965) Autoradiographic analysis of turnover times of normal and psoriatic epidermis. *Journal of Investigative Dermatology*, **45**, 257.

WEINSTEIN, G.D. & FROST, PH. (1968) Abnormal cell proliferation in psoriasis. *Journal of Investigative Dermatology*, **50**, 254.

ZAUN, H. & ALTMAYER, P. (1973) Ergebnisse reflexphotometrischer Bestimmungen der Vasoconstriction nach topischer Steroidapplikation. *Archiv für Dermatologische Forschung*, **247**, 379.

British Journal of Dermatology (1976) **94**, 263.

Protective action of beta-carotene against lethal photosensitization of fibroblasts *in vitro**

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SUMMARY

Cell culture experiments using haematoporphyrin photosensitized bovine hoof fibroblasts and long-wave uv-irradiation revealed two distinct and separable patterns of lethal photosensitization according to two different sensitization procedures:

(1) Photosensitization of cell membranes by short exposure (5 min) of cells to haematoporphyrin. (2) Cytoplasmic photosensitization elicited by a 2 h exposure of cells to haematoporphyrin. Cell membrane photosensitization was reversible by incubation of cells in serum which removed surface bound haematoporphyrin; cytoplasmic photosensitization was irreversible.

Beta-carotene was tested in these two systems and the following results were obtained: (1) Pre-incubation of bovine hoof fibroblasts in β-carotene protects from lethal haematoporphyrin photosensitization. (2) Protection with β-carotene is achieved against both types of photosensitization. (3) The protective effect of β-carotene depends upon the duration of pretreatment, reaching a maximum after 7 days. (4) β-carotene protection is maintained even after trypsinization of bovine hoof fibroblasts and withdrawal of β-carotene from the medium for 24 h or more. (5) Haematoporphyrin sensitized bovine hoof fibroblasts show a distinct pattern of red fluorescence for each type of photosensitization. Incubation of bovine hoof fibroblasts in β-carotene prior to haematoporphyrin photosensitization results in a pronounced reduction of red fluorescence. Some of these data indicate that β-carotene acts, at least in cell membrane photosensitization, at the level of the cell membrane into which it appears to be incorporated.

Mathews-Roth *et al.* (1970a, b) reported that β-carotene (bC) reduces photosensitivity in patients with erythropoietic protoporphyria (EPP) and this has been amply confirmed by subsequent studies (Mathews-Roth *et al.*, 1974; de la Faille *et al.*, 1972; Lewis, 1972; Chapel, Stewart & Webster, 1972; Gschnait & Wolff, 1974). The oral administration of bC has now become a safe and effective means of protecting these patients from the deleterious effects of long wave uv-light but the mechanisms by which this compound exerts its protective action remain unexplained. Since there is some evidence that bC acts at the cellular level (Swanbeck & Wennersten, 1973; Krinsky, 1968; Gschnait & Wolff, 1973; Sistrom, Griffith & Stanier, 1956; Mathews & Sistrom, 1960a, b) we decided to study bC in a cell culture model system in order to determine (1) its possible protective effect against haematoporphyrin-photosensitization in mammalian cells grown *in vitro*, and (2) the influence of photosensitization and bC exposure modalities on this protective action.

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MATERIAL

Cell line

A strain of fetal bovine hoof fibroblasts (BHF) was used in this study. It was isolated from fetal bovine hooves obtained under sterile conditions from a local slaughterhouse. The cells were grown in Falcon plastic flasks in Eagle's minimal essential medium with Hepes buffer and gentamicin, supplemented with 20% horse serum (Flow Laboratories). All experiments were performed during the logarithmic growth phase. There was no apparent change in cell morphology throughout this period (Fig. 1A).

Irradiation

A bank of four Philips blacklight tubes (TL 40W/08 RS) (uv), spaced 4·5 cm apart, was used as long wave uv-light source. The monolayers were irradiated in plastic culture flasks which were always positioned in the same area within the irradiation box. The operating distance was 12 cm. During irradiation the vessels were covered with 3 mm thick window glass. The uv energy which reached the cell surface was approximately 1000 μW/cm^2; the spectrum emitted was from below 320 to 400 nm with a peak at 355 nm. The uva output was measured with a long wave uv meter (Ultraviolet Products Inc., San Gabriel, California) and found to be constant throughout the experiments. A temperature of 37°C was maintained. Irradiation of 3 h and more did not produce degeneration of BHF monolayers.

Photosensitization

Haematoporphyrin (HP) (Calbiochem No. 3731) was used as photosensitizing agent as, in contrast to protoporphyrin, it is easily soluble in tissue culture media and its photosensitizing properties are comparable to those of protoporphyrin. HP was employed without further purification at a concentration of 2 mg/100 ml medium with 20% serum added. This was found to be non-toxic for BHB kept in the dark while it represented the lowermost concentration resulting in lethal photodamage after uv-irradiation. For purposes of fluorescence microscopy, HP was used at concentrations of 2–40 mg/100 ml medium.

Light microscopy

Monolayers grown in Falcon flasks and as coverslip cultures were viewed with Reichert Zetopan and Reichert Diavert phase contrast microscopes.

Fluorescence microscopy

Coverslip cultures were examined with a Zeiss Photomicroscope 1 using an Osram HBO 200 high pressure mercury lamp and Schott BG 12 excitation and 530 nm barrier filters.

β-carotene solution

bC is not soluble in aqueous media and the 'water-soluble' preparation of bC available to us (Hoffman

La Roche, Vienna) did not pass sterilization filters, when brought to suspension. To circumvent this difficulty we employed sera obtained from normal volunteers fed with bC (150 mg per day) for approximately 3 weeks. The bC was kindly provided by Hoffmann La Roche, Vienna. The bC levels of these sera ranged from 800 to 1000 μg%. The sera were heat inactivated and were mixed into Eagle's MEM with Hepes buffer and gentamicin at 75%. Final concentrations of bC in the media were within the range 700–800 μg%.

METHODS

Photosensitization procedures

As previously mentioned by Allison, Magnus & Young (1966), photosensitization of tissue culture cells can be due either to porphyrins residing on the cell surfaces or to intracytoplasmic porphyrins. Pilot experiments performed for the present study revealed that photosensitization of either type can be achieved selectively by using appropriate exposure times to HP and by taking advantage of the strong binding capacity for porphyrins of serum. The procedures listed below were found to elicit lethal phototoxic damage; the porphyrin concentrations and uv exposure times given represent the smallest doses and shortest exposures, respectively, that still yielded lethal phototoxicity.

Photosensitization by cell surface bound HP (Type I). Monolayers were exposed for 5 min to HP (2 mg/100 ml medium) in the dark, rinsed three times in serum free media to remove free HP and subjected to fluorescence microscopy or to uv-irradiation for 15 min to elicit photodamage.

Photosensitization by intracytoplasmic HP (Type II). Monolayers were incubated in HP medium for 2 h in the dark, rinsed three times and then incubated for 30 min in medium containing 20% horse serum to remove surface bound HP. Thereafter, they were either viewed with the fluorescence microscope or irradiated with uv-light for 40 min. Monolayers sensitized with 2 mg HP/100 ml medium and irradiated for 40 min still showed clear fluorescence in the fluorescence microscope. This indicated that there was a comparatively slow photochemical degradation of HP under the conditions used in our model system.

β-carotene experiments

Cultures were kept in medium containing bC from 1 h up to 3 weeks. After thorough rinsing in media (without serum) to remove all free bC, they were sensitized according to the type I and II sensitization procedures and subjected to fluorescence microscopy and uv-irradiation as stated above. The survival rates of the bC treated cultures as compared to non-treated controls were chosen as the main criterion of evaluation in these protection experiments. Cultures were examined by two investigators in a blind study fashion over an observation period of 4 days.

Control experiments

BHF were irradiated up to 3 h without photosensitizer; in addition they were kept in the dark for 24 h in the presence of 2 mg HP/100 ml growth medium, and bC treated BHF were cultured for 2 weeks. Under these conditions the cells grew very well, they did not show morphological changes as described later, and they exhibited largely the same growth pattern as compared to ordinary cultures or cultures in 75% human serum not containing bC (Fig. 1a).

Electron microscopy

Subcultures of type I and type II photosensitized BHF were trypsinized for 15 min using 0·2%

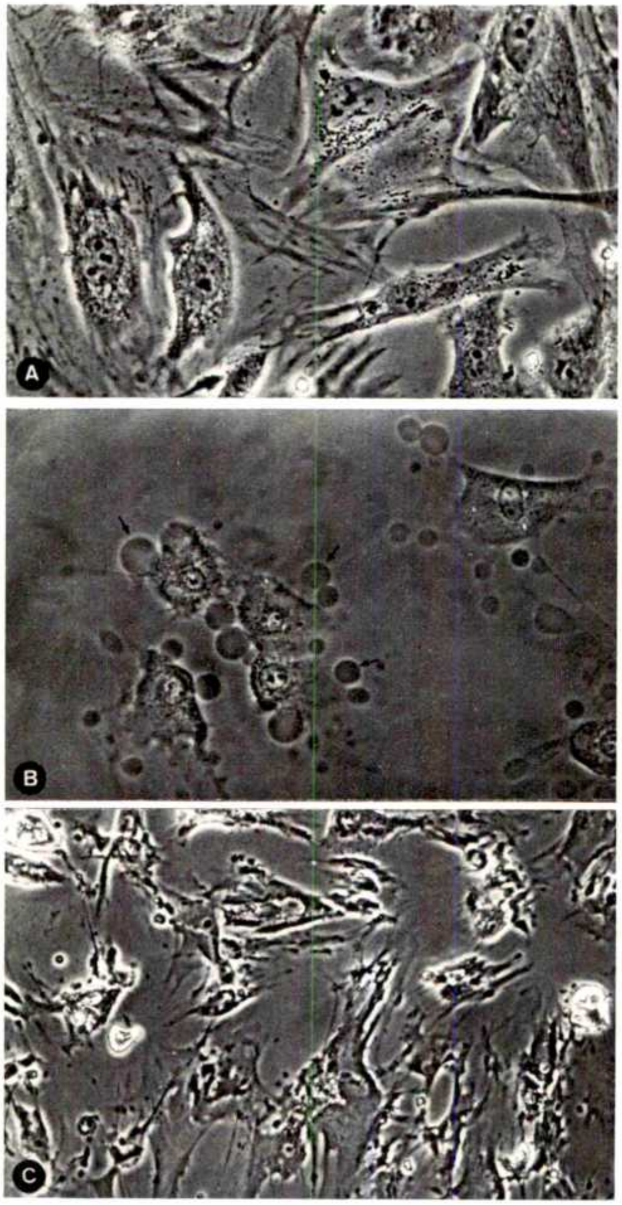

FIGURE 1. Bovine hoof fibroblasts, phase contrast (×250). (a) Untreated cells; (b) cell damage 2 h after type I sensitization and uv-irradiation. Note numerous cytoplasmic blebs (arrows); (c) cell damage 6 h after type II sensitization and uv-irradiation.

trypsin (Flow Laboratories), at 37°C and the cell suspensions were centrifuged at 1200 rev/min for 10 min in 2 ml plastic centrifuge tubes. The pellets were minced and fixed with a 1:1 dilution of the fixative described by Karnovsky (1965) for 3 h at room temperature, rinsed 3 times in 0·1 m cacodylate buffer, pH 7·2, at 4°C, postfixed at 0–4°C in 3% aqueous osmic acid and, after rapid dehydration in a graded series of alcohols, embedded in Epon 812. Ultrathin sections were cut with LKB ultrotome II and Reichert OM U2 ultramicrotomes, stained with lead citrate and examined with a Zeiss EM 9S electron microscope.

RESULTS

Photosensitization experiments

Phototoxic damage in type I sensitized cells

Cell damage was apparent after as short an irradiation as 15 min. It was heralded by the appearance of large numbers of bleb-like cytoplasmic sacs (Fig. 1B) which protruded from the cell surface, tended to enlarge, fused, and sometimes detached and floated off into the medium. A similar blebbing effect has been described previously by Allison *et al.* (1966). Two to 6 h after irradiation there was severe cytoplasmic vacuolization, pyknosis of nuclei, and rounding up of the cells. Six to 12 h after irradiation all of such damaged cells were dead. In experiments performed to substantiate further that in this type of sensitization HP resides on the cell surfaces only, monolayers were sensitized according to the type I procedure and carefully rinsed with serum-free media to remove non-cell bound HP. The monolayers were then incubated in serum containing media to bring all surface bound HP back into solution. If the wash was now used to sensitize a second set of monolayers according to the type I pattern and if both cultures were then subjected to uv-irradiation under identical conditions, only the second set of monolayers degenerated while the first ones remained unaltered.

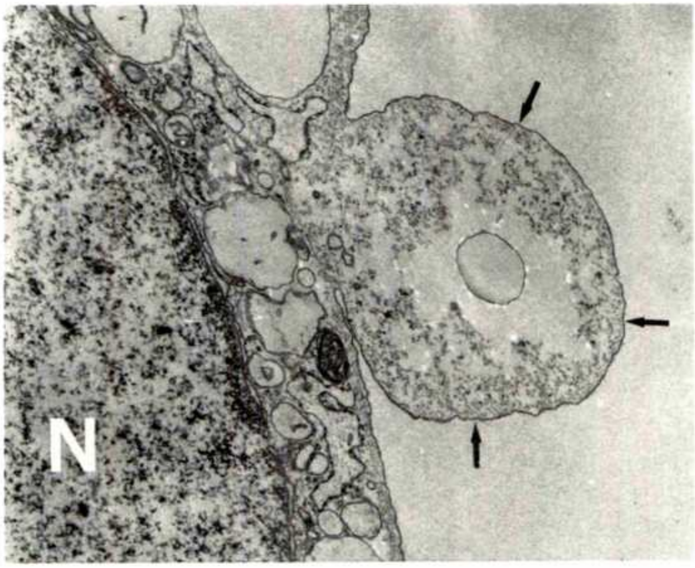

FIGURE 2. Electron micrograph of type I sensitized and uv-irradiated bovine hoof fibroblast. Note vacuolization of the cytoplasm. The cytoplasmic bleb (arrows) contains an electron lucent cytoplasm which appears peppered with dispersed ribosomes. N = nucleus (× 16,000).

Electron microscopy of type I photosensitized and irradiated cells revealed vacuolization and disorganization of the cytoplasm (Fig. 2). Mitochondria were swollen and sometimes disrupted. At the cell periphery, cytoplasmic blebs were frequently found which appeared as spherical protrusions delimited by a morphologically intact trilaminar cell membrane. They contained an electron-lucent, structureless cytoplasm which was well demarcated from the bulk of cellular constituents and appeared peppered with dispersed ribosomes.

TABLE 1. Type I sensitization (surface bound haematoporphyrin)

	No. of cultures		
	Survived	Died	Total
β-Carotene	17	1	18
Controls	2	13	15

Protection against phototoxic damage in type I sensitized cells. Monolayer cultures that had been incubated in bC medium for 1 week or more and subsequently sensitized according to the type I procedure showed significant protection as expressed by the survival rates at the end of a 4-day-observation period (Table 1). However, protection was not an all or nothing phenomenon as bC pretreated cultures also exhibited signs of degeneration that were morphologically similar though much milder in severity. At any given time of the observation period, the degenerative changes were less pronounced and involved a smaller number of cells which made the protected cultures easily distinguishable from the non-protected ones. Also, the phototoxic effects appeared considerably retarded: cytoplasmic blebs did not arise before 1–2 h after irradiation and after 6 h the majority of cells still looked reasonably healthy. Recovery usually occurred within 1 or 2 days in contrast to the non-protected cultures which were irreversibly damaged.

Phototoxic damage in type II sensitized cells

Degenerative changes became apparent approximately 2 h after irradiation. They manifested as blurring of the nucleus, disappearance of nucleoli, perinuclear arrangement of cell organelles and shrinking of the cell body (Fig. 1C). There were no alterations that could be considered characteristic for this type of cell injury. Cytoplasmic blebs were never observed. Cell death was complete after 12 h.

At the EM level such degenerating cells were characterized by multiple infoldings of the nucleus and severe vacuolization of the cytoplasm. The periphery of the cytoplasm appeared free of vacuoles and cell organelles but contained packed ribosomes and microfilaments. The cytoplasmic blebs observed in type I sensitized cells were absent.

Protection against phototoxic damage in type II sensitized cells. Again, protection was found in cultures exposed to bC for 1 week or more as estimated by the survival rates after the 4-day-observation period (Table 2). The protective effect manifested as retardation and attenuation of the degenerative changes which otherwise were morphologically identical to those in the non-protected controls. Recovery usually occurred after 2–4 days whereas non-protected cultures died.

TABLE 2. Type II sensitization (intracellular haematoporphyrin)

	No. of cultures		
	Survived	Died	Total
β-Carotene	9	1	10
Controls	2	7	9

Features of bC protection common to both cell surface (type I) and intracytoplasmic (type II) sensitization

Duration of bC pretreatment. Protective action was dependent on duration of exposure to bC medium. Pre-incubation of monolayers for a few hours prior to the HP–uv procedure resulted in virtually no protective effect. Protection increased with duration of bC pretreatment and reached its maximum after approximately 1 week. Further incubation failed to enhance protection.

Withdrawal of bC. The protective effect was not immediately abolished when bC was withdrawn from the cultures. It disappeared only slowly and was found to persist for some days after bC removal, in one run of experiments even as long as 2 weeks. This was also true for subcultures prepared from protected cultures by trypsinization and replanting.

Concentration of HP. The protective effect, even at the low HP concentrations chosen in our system, was not absolute and it was easily overcome by increasing the dose of HP to 3 or 4 mg/100 ml medium.

Fluorescence microscopy experiments

Type I sensitization resulted in a bright diffuse red fluorescence of the cells. Fluorescence was equally strong in all cells and extended homogeneously into the cell periphery except the nucleus. This type of fluorescence was made reversible by removing HP from the cells with serum. This finding corresponds to the reversibility of photosensitization as described in the section above.

Cells grown under bC for 1 week or more and subjected to type I sensitization exhibited a significantly weaker fluorescence when compared to untreated control cultures prepared under the same conditions. Most of the cells did not fluoresce at all, a minority of the population showed weak fluorescence, and only a few cells showed strong fluorescence that was comparable to that of non-protected controls.

Type II sensitization resulted in an intense bright red fluorescence of the perinuclear area of the cells whereas the cell periphery and the cell processes remained unstained. The nucleus and some intracytoplasmic vacuoles did not fluoresce and appeared as negative images.

Cultures grown in bC for 1 week showed a weaker fluorescence than did untreated control cultures. On the other hand, when type II sensitized cultures were mounted in bC serum and viewed with the fluorescence microscope they showed no impairment of fluorescence as compared to cultures mounted in plain serum.

Features of fluorescence common to surface bound (Type I) and intracytoplasmic (Type II) sensitization under the influence of bC. The above findings pertain only to cultures which had been grown in bC for at least 1 week. Simple pre-incubation in bC medium for a few hours produced only moderate or equivocal reduction of fluorescence. In analogy to the findings in the protection experiments, the effect of bC on fluorescence was not immediately abolished after withdrawal of bC from the medium. Even 1–2 weeks thereafter and even after trypsinization the cells failed to fluoresce as strongly and as uniformly as non-treated cells.

DISCUSSION

The protective effect of bC against photosensitization was recognized in studies in which the action of long-wave uv-light on pigmented and non-pigmented variants of bacteria was investigated (Sistrcm *et al.*, 1956; Mathews & Sistrom, 1960a, b). Mathews (1964) demonstrated protective activity of bC against photosensitization in mice that had been injected with haematoporphyrin and this lead to the introduction of this compound into the treatment of EPP (Mathews-Roth *et al.*, 1970a, b). Its beneficial effect in this disease is now well documented in more than seventy cases (Mathews-Roth *et al.*, 1974; de la Faille *et al.*, 1972; Gschnait & Wolff, 1974; Lewis, 1972; Chapel *et al.*, 1972).

The original observation on bacteria had already suggested the cellular level as site of action. Further data to support this view were provided by experiments that employed protoporphyrin (Swanbeck & Wennersten, 1973; Gschnait & Wolff, 1973), chlorpromazine and demethylchlortetracycline (Swanbeck & Wennersten, 1973) sensitized erythrocytes as an *in vitro* model. To our knowledge, the present report deals with the first attempt to study the effect of bC on viable cells in culture. The primary site of porphyrin induced uv damage in EPP appears to be the dermal capillary endothelium (Gschnait, Wolff & Konrad, 1975) but as endothelial cell cultures were unavailable to us at the time when the experiments were performed, bovine hoof fibroblasts were chosen as a model cell system.

Our findings demonstrate that the protective effect of bC can be achieved *in vitro* and that it occurs at the cellular level. However, it was unexpected to find that protection was similar in the two different types of HP-sensitization despite the apparent differences of cellular HP-localization and the differences in the morphological events and time curves of the phototoxic damage induced in the two sensitization procedures.

There can be little doubt that, in type I sensitization, HP was localized on or very close to the cell membrane. The evidence for this is three-fold: (1) Washing of type I sensitized cells with serum abolished their fluorescence and rendered them resistant to phototoxic damage. (2) HP washed from the cells with serum was transferred to other monolayers where it induced photosensitization. Since serum is unable to penetrate the cell membrane it appears reasonable to assume that in type I sensitized cells HP resided in or on the cell membrane. (3) The appearance of blebs as the hallmark of type I phototoxic damage (Allison *et al.*, 1966) also suggests involvement of the cytomembranes (Cooper, Goldring & Klein, 1962; Price, 1967; Hogue, 1919; unpublished data of this laboratory).

The interference of bC with type I bound HP—as manifested by a reduction of phototoxicity (Table 1) and a decrease of fluorescence—indicates that at least part of the action of bC was exerted on the cell membrane. This, in turn, suggests that bC was present at this site and there is some reason to believe that bC was in fact incorporated into the cell membranes: it required about one week of exposure to bC to secure the protective effect which persisted after withdrawal of bC from the medium and after trypsinization.

The nature of the experiments performed in this study precludes any conclusions as to the actual mechanisms by which photosensitized cells are protected by bC. As to type I photosensitization one could speculate that bC present in the cell membrane decreases the membrane's affinity for HP with the effect that phototoxicity and fluorescence are reduced. Alternatively, membrane-bound bC could exert a filtering function for long-wave uv at the cellular level; and lastly, it could quench singlet excited oxygen generated from the interaction of uv light and HP (Foote, Chang & Denny, 1970a, b) and prevent cholesterol peroxide formation within the cell membrane (Lamola, Yamane & Trozzolo, 1973). Similar interpretations can be profferred with regard to cytoplasmic photosensitization but the lack of supporting experimental data reduces their value to a speculative exercise. Clearly, further experimental work is needed.

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REFERENCES

Allison, A.C., Magnus, I.A. & Young, M.R. (1966) Role of lysosomes and of cell membranes in photosensitization. *Nature*, **209**, 874.

Chapel, T.A., Stewart, R.H. & Webster, S.B. (1972) Erythropoietic protoporphyria: report of a case successfully treated with carotene. *Archives of Dermatology*, **105**, 572.

Cooper, P., Goldring, I. & Klein, M. (1962) Induction of changes in surface activity of strain L cells at gas–membrane interphases. *Science*, **135**, 725.

De la Faille, H.B., Suurmond, N., Went, L.N., Stevenick, J. & Schothorst, A.A. (1972) Beta-carotene as a treatment for photo-hypersensitivity due to erythropoietic protoporphyria. *Dermatologica*, **145**, 389.

Foote, G.S., Chang, Y.C. & Denny, R.W. (1970a) Chemistry of singlet excited oxygen X. Carotenoid quenching parallels biological protection. *Journal of American Chemical Society*, **92**, 5216.

Foote, C.S., Chang, Y.C. & Denny, R.W. (1970b) Chemistry of singlet oxygen XI. Cis-trans isomerization of carotenoids by singlet excited oxygen and a probable quenching mechanism. *Journal of American Chemical Society*, **92**, 5218.

Gschnait, F. & Wolff, K. (1973) Erythropoietic protoporphyria. *European Society for Dermatological Research.* Amsterdam 1973, book of abstracts.

Gschnait, F. & Wolff, K. (1974) Die erythropoetische Protoporphyrie. *Hautarzt*, **25**, 72.

Gschnait, F., Wolff, K. & Konrad, K. (1975) Erythropoietic protoporphyria. Submicroscopic events during the acute photosensitivity flare. *British Journal of Dermatology*, **92**, 545.

Hogue, M.J. (1919) The effect of hypotonic and hypertonic solutions on fibroblasts of the embryonic chick heart *in vitro*. *Journal of Experimental Medicine*, **30**, 617.

Karnovsky, M.J. (1965) A formaldehyde–glutaraldehyde fixative of high osmolality for use in electron microscopy. *Journal of Cell Biology*, **27**, 137A.

Krinsky, I. (1968) The protective function of carotenoid pigments. *Photophysiology, current topics*, Vol. III (Ed. by A. C. Giese), p. 123. Academic Press, New York and London.

Lamola, A.A., Yamane, T. & Trozzolo, A.M. (1973) Cholesterol hydroperoxide formation in red cell membranes and photohemolysis in erythropoietic protoporphyria. *Science*, **179**, 1131.

Lewis, M.B. (1972) Effect of beta-carotene on serum vitamin A levels in erythropoietic protoporphyria. *Australian Journal of Dermatology*, **13**, 75.

Matthews, M.M. (1964) Effect of beta-carotene against lethal photosensitization by hematoporphyrin. *Nature*, **203**, 1092.

Mathews, M.M. & Sistrom, W.R. (1960a) The function of the carotenoid pigment in non-photosynthetic bacteria. *Nature*, **184**, 1892.

Mathews, M.M. & Sistrom, W.R. (1960b) The function of the carotenoid pigments in Sarcina lutea. *Archives of Microbiology*, **35**, 139.

Mathews-Roth, M.M., Pathak, M.A., Fitzpatrick, T.B., Harber, L.C. & Kass, E.M. (1970a) Beta-carotene as a photoprotective agent in erythropoietic protoporphyria. *New England Journal of Medicine*, **282**, 1231.

Mathews-Roth, M.M., Pathak, M.A., Fitzpatrick, T.B., Harber, L.C. & Kass, E.M. (1970b) Beta carotene as a photoprotective agent in erythropoietic protoporphyria. *Transactions of American Physicians*, **83**, 176.

Mathews-Roth, M.M., Pathak, M.A., Fitzpatrick, T.B., Harber, L.C. & Kass, E.M. (1974) Beta-carotene as an oral photoprotective agent in erythropoietic protoporphyria. *Journal of American Medical Association*, **228**, 1004.

Price, Z.H. (1967) The micromorphology of zeiotic blebs in cultured human epithelial (Hep) cells. *Experimental Cell Research*, **48**, 82.

Sistrom, W.R., Griffith, M. & Stanier, R.Y. (1956) Biology of a photosynthetic bacterium which lacks colored carotenoids. *Journal of General Physiology*, **48**, 473.

Swanbeck, G. & Wennersten, G. (1973) Effect of beta-carotene on photohemolysis. *Acta dermato-venerologica (Stockholm)*, **53**, 283.

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Prostaglandins and pruritus*

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SUMMARY

We have found that pretreatment of human skin with prostaglandin E_1 significantly lowers the threshold of human skin to itching evoked by both histamine and papain. Prostaglandins may thus potentiate pruritus in inflammatory skin disease, presumably by a non-specific effect on nerve-endings.

The cause of itching in inflammatory skin disease is unknown. Prostaglandins are known to be present in increased amounts in inflamed skin (Angaard, Arturson & Jonsson, 1970; Greaves & Sondergaard, 1970; Greaves, Sondergaard & McDonald-Gibson, 1971; Arturson, Hamberg & Jonsson, 1973), and Greaves & McDonald-Gibson (1973) have produced evidence that a prostaglandin may contribute to pruritus by lowering the itch threshold of skin. We have now tested this idea further by determining the threshold to histamine-evoked and papain-evoked itching in normal skin and in skin pre-treated with prostaglandin.

PATIENTS AND METHODS

We studied ten male and ten female subjects (age range 17–65 years) who were either volunteer members of the medical staff or patients with localized non-inflammatory skin disease such as warts. None of the subjects was taking antipruritic or anti-inflammatory drugs at the time of the study.

The effect of prostaglandin E_1 on the threshold of the itch induced by histamine and papain was studied in the following way. Both forearms were washed and dried, and four 1 cm^2 areas were lightly scarified on the flexor surface of each forearm, care being taken to avoid any bleeding. A 1 cm^2 piece of gauze soaked in prostaglandin E_1 in a concentration of 1 μg/ml was placed on two scarified areas on each forearm and left for 30 min. As control sites the other two scarified areas on each forearm were treated with gauze soaked in phosphate-buffered saline. The allocation of prostaglandin or saline to the four sites on the right arm was randomized and the opposite allocation was made to the four sites on the left arm, so that symmetrical paired comparisons between prostaglandin and saline-treated areas could be made. The itch threshold for histamine was then determined in two of the paired areas, and the threshold for papain was determined in the other two paired areas. The test solutions used were histamine acid phosphate (1 mg/ml, expressed as histamine base) and twice crystallized papain suspension containing 16–40 BAEE units/mg (Sigma London Chemical Co. Ltd.). Five minutes after removal of the gauze, 0·1 ml of histamine diluted 1:1000 was placed on the scarified skin and left for 2 min. This was followed at 2 min intervals after drying by increasing concentrations of histamine (1:500, 1:200, 1:100, 1:50, 1:20, and 1:10) until itching occurred. The itch threshold was recorded as the lowest concentration of histamine which produced itching. The itch threshold for papain was similarly determined, starting with a 1 in 500 dilution of the papain solution and progressing by geometric increments to a 1 in 5 dilution. In each patient two paired comparisons of saline-treated and prostaglandin-treated sites were thus made for both histamine and papain and both observer and patient were unaware of the allocated treatment.

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* Based on a paper read at the 1975 Meeting of the Investigative Group of the B.A.D.

RESULTS

In the concentration used prostaglandin E_1 did not by itself cause itching after application to scarified skin. We found the results expressed as a dilution to be log-normal. To normalize the distribution, therefore, we have expressed our data as the logarithm of the lowest concentration which produced itching (Fig. 1 a and b). These results show that, although there is a wide scatter, there is a tendency for the prostaglandin-treated side to have a lower threshold for pruritus evoked both by histamine and papain. To enable the statistical significance of this change to be assessed we calculated the mean log ratio (dilution for prostaglandin side/saline side) of the two matched pairs of tests for each patient, for both histamine and papain. The mean of these mean log ratios for the twenty patients was 0·1001 ± 0·045 for histamine and 0·1100 ± 0·050 for papain. That is, on average the concentration of histamine on the prostaglandin-treated side was 0·80 times that on the saline-treated side, and for papain the figure was 0·78. Both the mean log ratios were significantly greater than zero (t = 2·224 and 2·200 respectively, d.f. = 19, $P < 0·05$), and this suggests that treating the skin with prostaglandin had significantly potentiated the pruritic effect of both histamine and papain.

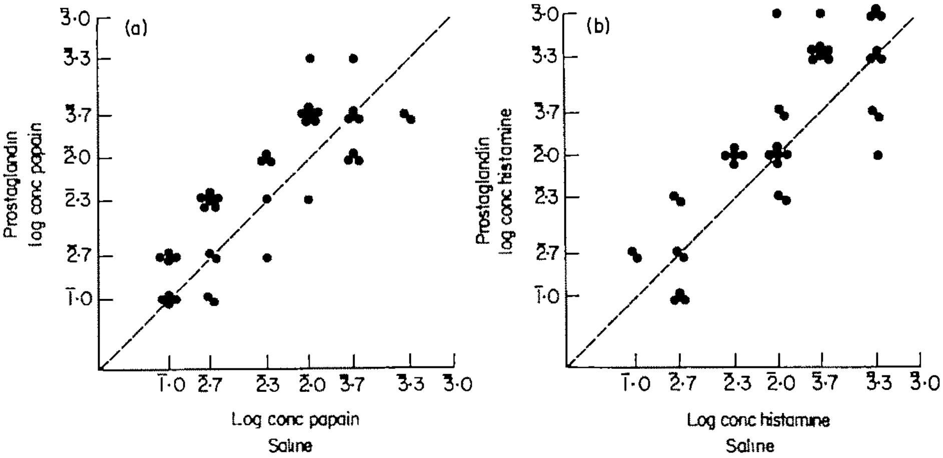

FIGURE 1. Histamine (a) and papain (b) thresholds in skin pretreated with normal saline and with prostaglandin. Each result is expressed as the log of the first dilution which produced itching, and there are two results from every patient in each figure.

DISCUSSION

Our results show that prostaglandin E_1 lowers the threshold of human skin to itching evoked by both histamine and papain. This supports the conclusion of Greaves & McDonald-Gibson (1973) that prostaglandins synthesized locally in inflamed skin, though not themselves pruritogenic, might sensitize the nerve receptors to the effect of other pruritogens. The methodology of their study of the effect of prostaglandin E_1 on histamine-induced itch in twenty-three subjects was similar to ours, but they reported that prostaglandin produced an effect of a much greater statistical significance ($P < 0{\cdot}005$) than we have found. This may be attributed partly to variation in potency of prostaglandin E_1 obtained from different sources. A further factor which may have spuriously enhanced the statistical significance of their results is that they compared the mean itch threshold on the treated and control sites in terms of histamine concentration without attempting to normalize the results. The application of Student's '*t*'-test to such skew distributions is inappropriate, and is likely to produce a spuriously high significance. Nevertheless our present work confirms their general conclusion that prostaglandin E_1 potentiates histamine-evoked itch, and we have also extended their observations to show that the effect is not specific to histamine but applies also to papain-evoked itch. It seems likely, therefore, that this may be a general effect of prostaglandins on the perception of itch. This idea is supported by the observation that prostaglandins cause cutaneous hyperalgesia (Solomon, Juhlin & Kirschenbaum, 1968; Ferriera, 1972), since itch and pain perception are closely related (Keele & Armstrong, 1964). The biological importance of this potentiating effect of prostaglandin on the production of itch and pain is unclear, but it may explain the old observations that the sensitivity to noxious chemical stimulation is greatly enhanced by the presence of inflammation (Whytt, 1968, Wolf & Wolff, 1943; Landan & Bishop, 1953).

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REFERENCES

ANGAARD, E., ARTURSON, G. & JONSSON, C.-E. (1970) Effect of prostaglandins in lymph from scalded tissues. *Acta Physiologica Scandinavica*, **80**, 46A.

ARTURSON, G., HAMBERG, M. & JONSSON, C.-E. (1973) Prostaglandins in human burn blister fluid. *Acta Physiologica Scandinavica*, **87**, 270.

FERREIRA, S.H. (1972) Prostaglandins, aspirin-like drugs and analgesia. *Nature: New Biology*, **240**, 200.

GREAVES, M.W. & MCDONALD-GIBSON, WENDY (1973) Itch: role of prostaglandins. *British Medical Journal*, **iii**, 608.

GREAVES, M.W. & SONDERGAARD, J. (1970) Pharmacologic agents released in ultraviolet inflammation studied by continuous skin perfusion. *Journal of Investigative Dermatology*, **54**, 365.

GREAVES, M.W., SONDERGAARD, J. & MCDONALD-GIBSON, WENDY (1971) Recovery of prostaglandins in human cutaneous inflammation. *British Medical Journal*, **ii**, 258.

KEELE, C.A. & ARMSTRONG, D. (1964) *Substances Producing Pain and Itch*, pp. 10–29. Arnold, London.

LANDAU, W.M. & BISHOP, G.H. (1953) Pain from dermal, periosteal and fascial endings, and from inflammation. *A.M.A. Archives of Neurology and Psychiatry*, **69**, 490.

SOLOMON, L.M., JUHLIN, L. & KIRSCHENBAUM, M.B. (1968) Prostaglandin on cutaneous vasculature. *Journal of Investigative Dermatology*, **51**, 280.

WHYTT, R. (1968) *The Works of Robert Whytt*, pp. 255–306. Becket and Dehoudt, London.

WOLF, S. & WOLFF, H.G. (1943) *Human Gastric Function: An Experimental Study of a Man and his Stomach.* Oxford University Press, London.

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The syndrome of ankyloblepharon, ectodermal defects and cleft lip and palate: an autosomal dominant condition

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SUMMARY

Seven patients from four families are reported who had an inherited condition of which the main features were ankyloblepharon, ectodermal defects and cleft lip and palate. The ectodermal defects were partial or complete hair loss, absent or dystrophic nails, pointed widely spaced teeth and partial anhidrosis. Associated anomalies included lacrimal duct atresia, supernumerary nipples, syndactyly and auricular deformities. The inheritance of this abnormality was consistent with that of an autosomal dominant trait. The relationship between this and similar syndromes is discussed.

Ankyloblepharon, or fusion of the eyelids, is rarely complete. The lids are usually joined at birth by a solid band at the lateral or medial margins or by numerous epithelial strands. In the latter instance the condition is named ankyloblepharon filiforme adnatum, and the strands may contain elements of vascular and connective tissue. Where genetic evidence is available, the inheritance of ankyloblepharon has been described as that of an autosomal dominant condition (Sorsby, 1970). A genetic aetiology for ankyloblepharon filiforme adnatum is less certain, but it has been described in conjunction with cleft palate and lip (Long & Blandford, 1962).

Genetically determined defects of ectodermal tissues have long attracted medical attention because of the bizarre appearances produced. Cockayne (1933) divided these disorders into two genetically distinct groups, the anhidrotic and the hidrotic ectodermal dysplasias, whose distinguishing features were the presence or absence of sweating, nail or dental defects. The anhidrotic form was X-linked and hidrotic ectodermal dysplasia was inherited as an autosomal dominant condition.

There is no doubt that there are defects of other ectodermally derived tissues which may occur in isolation. These include both alopecia and anonychia. Again, there is genetic heterogeneity and amongst them there are some autosomal dominant varieties.

The anomaly of cleft lip and palate is relatively common and may be due to many different causes.

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For example, it is known that teratogens may produce the condition, but it can also be inherited. An isolated cleft palate is under multifactorial (polygenic) influence. However, when cleft lip and palate are inherited in association with another abnormality, for example with congenital sinuses of the lower lip (Michaelides, Hay & Wells, 1975), the inheritance is often that of an autosomal dominant type. This is probably the case with the individuals described below.

We are reporting seven individuals from four families who showed a combination of these three abnormalities which does not appear to have been described before and which was probably inherited as an autosomal dominant condition.

Ascertainment

The four probands were referred to the Genetic Clinic at St John's Hospital for Diseases of the Skin or to the Hospital for Sick Children, Great Ormond Street. One patient (case 1) has been presented at a meeting of the Dermatology Section of the Royal Society of Medicine (Hay & Wells, 1975). Three of the individuals had been under the care of other dermatologists, before they were referred to us, and we are grateful to them for their permission to study these patients.

Method of investigation

Pedigree details (Fig. 1) were recorded at the first visit of each patient and a complete clinical examination was carried out. Where possible their families were visited in their homes and all were examined.

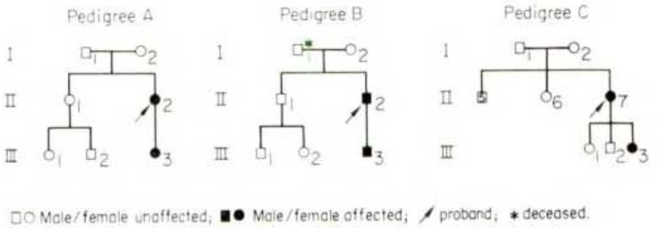
Pedigree A
Pedigree B
Pedigree C
I
II
III
□○ Male/female unaffected; ■● Male/female affected; ⁄ proband; * deceased.

FIGURE 1. Pedigrees A, B and C.

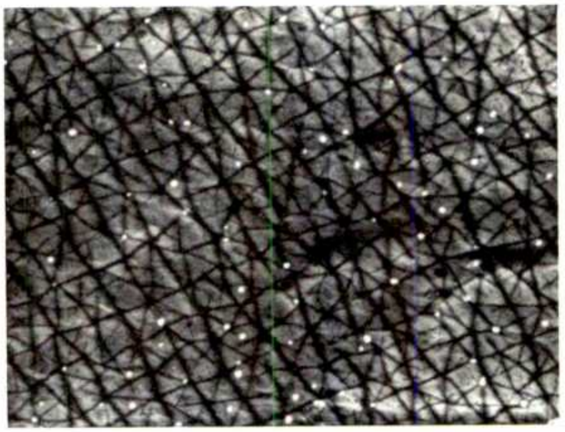

FIGURE 2. Silflo impression from an affected patient. The number of sweat pores seen is reduced compared with normal controls.

TABLE 1. Results of sweating tests (in active pores/cm^2)

	Conditions of stimulation	Flexor aspect of forearm	Hypothenar eminence	Fingertip	Forehead	Upper chest (anterior)	Axilla
Case 1	a) Rest	0	8	86	—	—	—
	b) Heat	94	12	120*	83	21	20
	c) Iontophoresis	122	40	—	—	24	—
Case 3	a) Rest	2	5	10	—	—	—
	b) Heat	16	16	20	80	48	14
	c) Iontophoresis	30	8	—	—	31	—
Case 7	a) Rest	0	22	60	—	—	—
	b) Heat	20	64	86	108	10	10
	c) Iontophoresis	41	54	—	—	20	—
Normal controls	a) Rest	0	8	5	—	—	—
(average results	b) Heat	120	200	140	190	120	110
of 3 controls)	c) Iontophoresis	158	180	—	—	112	—
Normal controls after Kuno (1956)	Heat	100–190	140–400	—	200–400	100–160	100–210

* 104 pores/cm^2 active 20 min after cessation of thermal stimulus.

Three patients (cases 1, 3 and 7) were admitted to Guy's Hospital so that further studies could be performed.

Sweating was evaluated by an impression technique using a silicone material, Silflo. Counts of active sweat glands from six areas (Table 1) were recorded first, at rest; second, after iontophoresis of an aqueous solution of 0·5% pilocarpine; and third, after a hot bath. Casts were taken by spreading a fine layer of the Silflo resin over the skin in the areas tested and the resulting impression was then projected onto a screen through a 1 cm square grid. The area over each gland producing sweat showed up as a point of light (Fig. 2). These points could be counted and an approximate estimate of their size made. Finally a direct pore count was made of the impression under a dissecting microscope equipped with a micrometer scale. The use of similar methods in hidrotic ectodermal dysplasia and other conditions has been previously described (Harris, Polk & Willis, 1972).

CLINICAL FEATURES

Pedigree A

Case 1. II.2 *G.H. Female, aged* 27 *years.* This patient was born at full term after a normal delivery. The birth weight was not known. At birth her eyelids were fused by epithelial strands at the lateral lid margins and were divided surgically. A median cleft palate and cleft lip on the right were also repaired shortly after birth. Her hair had always been sparse and was shed progressively through childhood. Hair fall was often exacerbated by periods of scalp infection. Her nails had been dystrophic since birth. Her teeth were poorly formed and pointed and had been removed by the age of 12 years. There was no specific difficulty with sweating. In addition, she had experienced recurrent episodes of photophobia and lacrimation.

Family history. There was no family history of a similar disorder or of its components and there was no history of consanguinity. Her daughter is case 2.

On examination. The patient was an adult female of normal bodily proportions. However, she had a striking facies, with broadened nasal bridge and hypoplastic maxilla. There was an incompletely repaired median cleft palate. Her chin was prominent.

The scalp hair was almost totally absent. Some of her nails were dystrophic, and the abnormality varied in extent from total absence to a terminal dystrophy. Her skin was dry and relatively hairless, particularly in the axillae and pubic areas. There was a supernumerary nipple on each anterior axillary fold and partial syndactyly of the second and third toes. Her eyes were injected and the lashes were short and incurved. No lacrimal puncta could be discerned on either upper or lower eyelid on either side. There was palmar and plantar hyperkeratosis with obliteration of dermatoglyphic patterns.

Her cardiovascular, nervous, respiratory and genital systems were all normal on clinical examination.

Case 2. III.3 *M.H. Female, aged* 2 *years.* This patient is the daughter of case 1. Her birth weight was 3·2 kg. Like her mother, her eyelids were fused at the lateral margins by short bands. She was born with a cleft palate but no hare lip. From early childhood, her scalp hair was sparse, fair and wiry with some patches of alopecia where her scalp had been damaged by recurrent bacterial infections. She had dystrophic finger nails, but normal toe nails. There was no history of heat intolerance or recurrent colds.

On examination. Her facial structure was difficult to evaluate because of her age. However, there was a posterior median cleft of the palate. Few teeth had erupted and those present were irregular and pointed. Her skin was dry. Her eyes were injected and there was a marginal blepharitis. Also the lacrimal puncta were small and atrophic. There was no palmar or plantar hyperkeratosis.

Pedigree B

Case 3. II.2 *K.C. Male, aged* 38 *years*. This patient was born 3 weeks prematurely. His birth weight was 2·9 kg. It was not known if his eyelids were fused at birth. However he had a cleft palate without cleft lip. His hair, including eyebrows and eyelashes, had always been sparse. Finger nails had never formed and only the big toe nails on his feet. He never developed a full second dentition and his teeth were irregular in shape and carious from an early age. There was no heat intolerance. Recently he had had attacks of photophobia and lacrimation. Also, he was partially deaf in the left ear, with an onset during the last 10 years.

Family history. There was no relevant history of skin problems but his mother was deaf in the left ear. No further details of this deafness were available. His only son is case 4.

Medical history. He reported that he was treated for hypothyroidism in early childhood, but no record of this was available. He had also been investigated for right bundle branch block, discovered on a routine E.C.G. examination, but no cause was found. A benign granuloma was removed from his vocal cords in 1972.

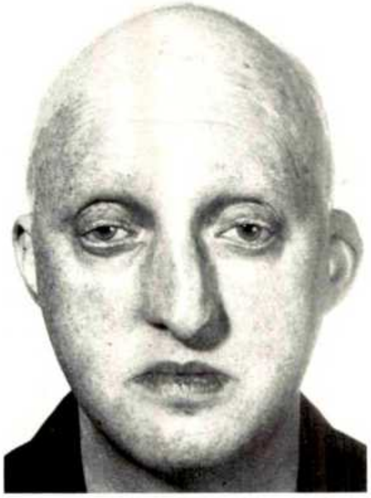

FIGURE 3. Frontal view demonstrating the facies, alopecia and ear deformity.

On examination. His facies (Fig. 3) was strikingly similar to case 1, with a broadened nasal bridge and sunken maxilla (Fig. 4). Again an incompletely repaired median cleft palate was noted. His teeth, which were widely spaced, had been extensively filled and capped. Finger (Fig. 5) and toe nails, apart from the big toe nails, were absent. His skin was dry with reticulate hyperpigmentation prominent on the forearms and in the axillae.

His conjunctivae were injected and he was severely photophobic, but he reported that this tended to fluctuate. There was bilateral arcus senilis. There was a partial neural hearing loss in the left ear and

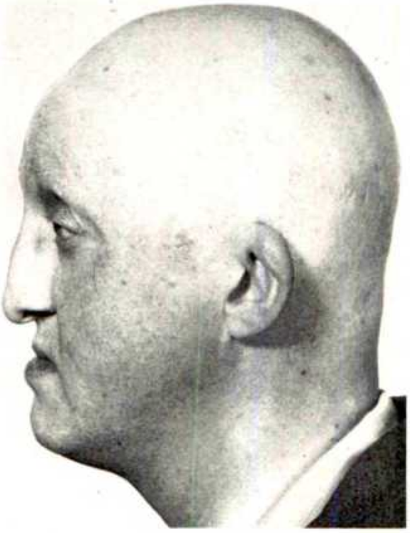

FIGURE 4. Lateral view. Loss of prominence of maxilla is shown.

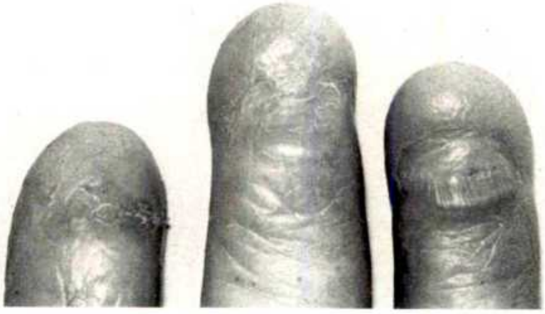

FIGURE 5. Close up view of middle three fingers of the right hand showing the loss of nail substance.

the external ear on this side was deformed and cup-shaped. His voice was hoarse. The palms and sole were hyperkeratotic and cracked.

The cardiovascular, nervous, respiratory and genital systems were normal on examination.

Case 4. III.3 *M.C. Male, aged* 1 *month*. This patient is the only son of case 3. His birth weight was 3·9 kg. He was born with fusion of the lateral lid margins and with a median cleft palate. Finger and toe nails were absent.

Family history. His mother had idiopathic epilepsy, diagnosed in childhood, and was being treated with phenytoin, including during the time she was pregnant.

On examination. There was a large mid-line cleft of the palate and moderate micrognathus was noted. One canine tooth had erupted. However, his hair was sparse and fair. There was a resolving cephalhaematoma and cradle cap eczema associated with a folliculitis. In addition, his eyelashes were sparse and there were no finger or toe nails. The skin on the legs and head was dry. There was partial ankyloblepharon filiforme adnatum more extensive on the left eye than the right.

No other abnormalities were detected apart from a mild and asymptomatic phimosis.

Pedigree C

Case 5. II.7 *R.W. Female, aged* 32 *years.* This patient weighed 2·0 kg at birth and delivery was normal: she was born with a cleft palate. Her hair was dark at birth but was shed rapidly. Subsequently there was some patchy regrowth with coarse fair hair, but this too was lost at puberty. She never had pubic or axillary hair and eyelashes were always sparse. Finger and toe nails were severely dystrophic. Both primary and secondary dentition were incomplete and irregular. The latter consisted of pointed teeth and many did not erupt. All teeth were shed by the age of 15 years. She had had attacks of photophobia in the past, but had never had treatment.

Family history. A maternal cousin had a cleft lip and palate, but without any other defects. No other relatives were affected, apart from her daughter (case 6).

Past medical history. She reported that small nodules were removed from her eyelid margins when she was a child, but no detailed records were available.

On examination. She had the characteristic facies of the other adult members of this group, with a broad flat face. The repair of a median cleft of the palate was noted and her teeth were absent. Scalp hair was almost totally absent and her nails were all dystrophic and brittle. Her skin was dry.

She had a chronic blepharitis, but all lacrimal duct puncta were visible. In addition there was palmo-plantar hyperkeratosis with partial obliteration of the dermatoglyphic patterns.

Case 6. III.3 *S.W. Female, aged* 1 *year.* This patient is the daughter of case 5. Her birth weight was 3·1 kg. and the confinement was normal. She was born with a cleft palate. Ankyloblepharon from the lateral border up to the mid-line was divided surgically shortly after birth. However, her nails at birth were rudimentary. Subsequently she had developed two teeth, which were discoloured and irregularly pointed. Sweating was normal and there was no heat intolerance.

On examination. There was a large mid-line cleft of the palate. The hair, nails and teeth were all dystrophic. There were no visible lacrimal puncta on either side and the lid margins were inflamed. In addition, there was an epicanthal fold on the right side.

There were a number of filamentous bands joining the lateral walls of the posterior aspect of the vagina. There was also a posterior anal fissure.

There was no abnormality of the cardiovascular, nervous or respiratory systems.

Case 7. *L.M. Female, aged* 10 *years.* (*Pedigree not illustrated.*) This patient's birth weight was 2·4 kg and she was 4 weeks premature. Her eyelids were fused at birth but spontaneous separation took place. There was a cleft palate but no cleft lip. Her hair was sparse and brittle. There were no nails at birth and subsequent growth was poor and dystrophic. Primary dentition had been retained. She had no photophobia.

Family history. There was no family history of any relevant disorder. Consanguinity was excluded

Past medical history. Her choanal atresia was corrected surgically in 1964. The cleft palate and mandibular prognathism were repaired in 1966.

On examination. This patient had the same characteristic facies with a hypoplastic maxilla and oval face. There was a central palatal defect with a surrounding rim of oral candidiasis, secondary to a palatal prosthetic plate. Her teeth were discoloured, carious and pointed, but all were present. Her hair was coarse and sparse, particularly in the occipital area, and there were some white hairs scattered anteriorly. Her nails were severely dystrophic. The skin was smooth, dry and relatively hairless. There was partial syndactyly of both second and third toes and of the third and fourth toes on the left (Fig. 7). There was also partial syndactyly of the third and fourth fingers bilaterally. Her left ear was deformed, with a cup-shaped auricle.

The intercanthal distance was 32 mm and this is within the definition of telecanthus. There was

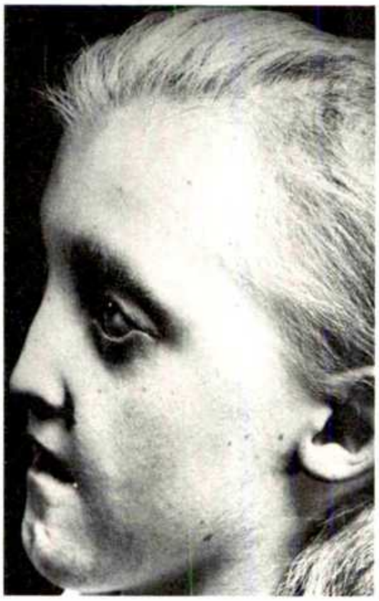

FIGURE 6. Lateral view of the face. Note the retention of partial ankyloblepharon of the left eye.

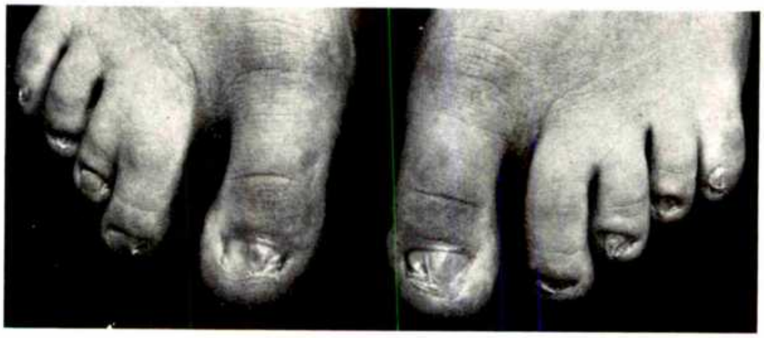

FIGURE 7. A view of the toes to illustrate the degree of syndactyly.

partial ankyloblepharon on the lateral lid margin of the left eye (Fig. 6). Upper lacrimal duct puncta were present, although the orifice on the left was reduplicated. Orifices on both lower lids were small and atrophic.

No other abnormality was seen.

RESULTS

Sweating tests were carried out as described above on cases 1, 3 and 7. The results are summarized in Table 1.

In Case 1 there was a patchy loss of glands over most of the areas tested. The best response was on the hands to a thermal stimulus and large amounts of sweat were produced from fewer glands than normal. Sweat was produced for at least 20 min after cessation of the stimulus, whereas in the normal controls it stopped promptly. In cases 3 and 7 a similar patchy loss of sweating was seen, although the response on the hands was not as dramatic. The results can be interpreted as a net loss of sweat glands, although the distribution of this loss was dissimilar in the patients tested.

Hair shaft microscopy. Samples of hair from three of the patients were examined both in longitudinal profile and in cross-section under the light microscope. The hairs were of a relatively wide diameter and showed actual rotation at various points along the hair shaft. In the cross-section the hair cuticles were either thinned or absent, even though the sections were performed at a proximal point on the hair shaft. The shape of most of the hairs was markedly abnormal. There were multiple concavities on the perimeter of the hair which presented a scalloped appearance, as opposed to the smooth round or oval shape of normal hairs. Scanning electron microscopy confirmed the presence of a frequently defective cuticle and also showed longitudinal fluting of the hair shaft.

The appearances presented by these hairs were identical to those seen in hereditary hypotrichosis (Marie-Unna Type) (Hutchinson & Wells, 1975).

Other investigations

Cases 1, 3 and 7 were admitted for investigation to Guy's Hospital. This included estimations of haemoglobin, white cell count, E.S.R., R.P.C.F.T. and V.D.R.L., plasma proteins, liver function tests and blood sugar. All were normal. In case 3, serum lipids and thyroid function tests were studied because of the arcus senilis and the past history of hypothyroidism. Both these were normal.

In addition, skull and chest X-rays were performed on these patients. These were also normal, although the skull films demonstrated the palatal defect.

An E.C.G. was performed on case 3 and this showed a right bundle branch block without any other evidence of cardiac pathology. An E.E.G. performed on case 7 at another hospital 4 years previously was reported as showing an area of dysfunction in the anterior part of the left hemisphere.

A skin biopsy was taken from the axilla of case 3 and showed almost total absence of epidermal appendages. There were no other abnormal histological features apart from increased melanin deposition in the basal layer in part of the section.

Slit lamp examination of the eyes of cases 1, 3 and 7 was carried out after instillation of oculent fluorescein. Only case 3 showed evidence of keratitis. However, in case 1 there was no excretion of the dye into the nasal or oral cavities. This supported the clinical impression of atresia of the lacrimal ducts.

The intelligence of all the patients studied was normal on gross testing.

Genetic findings

The first three pedigrees are illustrated (pedigrees A, B and C, Fig. 1). The inheritance of this

TABLE 2. Comparison of the features of the A.E.C. and similar syndromes

	Anhidrotic ectodermal dysplasia	Hidrotic ectodermal dysplasia	Ectrodactyly ectodermal defects and clefting (E.E.C. syndrome)	Ankyloblepharon ectodermal defects cleft lip and palate (A.E.C. syndrome)
Inheritance	X-linked recessive	Autosomal dominant	Autosomal dominant	Autosomal dominant
Heterozygous effects	Carrier females may show hypohidrosis, hypodontia, etc.	—	—	—
Skin	Dry	Palmar/plantar keratoderma	Dry palmar/plantar keratoderma	Dry palmar/plantar keratoderma
Hair	Partial to total alopecia	Partial to total alopecia	Partial alopecia	Partial to total alopecia
Nails	Some dystrophy	Severe dystrophy	Dystrophic	Severe dystrophy
Teeth	Severe hypodontia	Minor dental ailments common	Partial anodontia	Severe hypodontia
Sweating	Absent (?extent)	Present (?extent)		Reduced
Mental retardation	Nil	Nil	Only with recessive variety	Nil
Associated defects	Syndactyly Hyperpigmentation Sensory-neural deafness Lacrimal duct atresia Deformed ears	Syndactyly Photophobia Lens opacities	Syndactyly Lacrimal duct atresia Deformed ears	Supernumerary nipples Hyperpigmentation Syndactyly Photophobia Lacrimal duct atresia Deformed ears

disorder was compatible with that of an autosomal dominant trait, because both sexes were affected equally severely. Parents and children were affected rather than siblings alone. The father to son transmission in pedigree C excluded X-linkage. The four probands were presumably the first recipients of a new abnormal mutation. There was no consanguinity.

In the third family (cases 5 and 6) there was a maternal cousin who had a cleft lip and palate. He now lives abroad and could not be examined. The significance of this observation was hard to evaluate. There was no evidence in the other families of independent segregation of characters and it was possible that this one case was either a non-genetic variant or the unconnected expression of this relatively common genetic condition on its own.

DISCUSSION

Differential diagnosis. The features common to all the patients were ankyloblepharon, ectodermal defects and cleft lip and palate. Ankyloblepharon was present in all patients apart from two (cases 3 and 4) in the form of ankyloblepharon filiforme adnatum. In case 4 it is possible that the tumours on the eyelids removed in childhood were the remnants of this defect. Ectodermal defects, such as alopecia, hypodontia and nail dystrophy, were present in all cases. Cleft palate alone occurred in all families apart from the second group (pedigree B) where a cleft lip was present, in addition, in the proband.

Bowen & Armstrong (1973) described a genetically distinct but similar syndrome in which these defects occurred in two siblings in association with mental retardation. The inheritance was of an autosomal recessive type. However, ectodermal defects of comparable severity and inheritance have been described in association with cleft lip and palate alone (Rapp & Hodgkin, 1968). Two other similar conditions have been recorded. One, the E.E.C. syndrome (or ectrodactyly, ectodermal defects and clefting syndrome) has been described by a number of authors (Rudiger, Haase & Passarge, 1970; Brill, Hsu & Hirschhorn, 1972). The features of this syndrome are contrasted with those of our cases, and anhidrotic and hidrotic ectodermal dysplasia in Table 2. The other syndrome, the L.A.D.D. syndrome, the main features of which are lacrimal duct atresia, auricular deformities, dental and digital anomalies, covers a similar range of defects (Hollister *et al.*, 1973). All these conditions may be inherited as autosomal dominant traits, although, when the E.E.C. syndrome was associated with mental retardation, the inheritance was autosomal recessive (Freire-Maia, 1970).

Ectodermal defects. The ectodermal defects in this syndrome share important features in common with the classical anhidrotic and hidrotic ectodermal dysplasias (Table 2). The presence of sweating, in reduced amounts, has already been noted in patients with features typical of anhidrotic ectodermal dysplasia (Everett *et al.*, 1952), although this belies the description 'anhidrotic'. Until more satisfactory methods are available for evaluating the structure, distribution and response of sweat glands, this difficulty in defining the type of sweating abnormality will persist. It is likely that the distinction between the anhidrotic and hidrotic conditions, as separate phenotypes, is not as precise as was originally thought. However, the genetic differences remain and are extremely valuable as a guide to prognosis and inheritance.

Cockayne (1933) reported a rare dominant form of anhidrotic ectodermal dysplasia and Kerr, Wells & Cooper (1966) discussed patients with this diagnosis, but the existence of this type has still not been substantiated.

The associated abnormalities (Table 2) in our patients, such as syndactyly and supernumerary nipples, have all been described before in association with the ectodermal dysplasias. This is a further example of the similarities to be found in this group of disorders.

It was difficult to find an adequate explanation for the characteristic facies of our patients. However, similar features have been described in patients with cleft palates and in their unaffected relatives (Frazer & Pashayan, 1970). For instance, our group and theirs shared underdeveloped maxillae and a trapezoid facial shape. Hence the existence of a cleft palate in our patients may explain their facies.

Embryological aspects. Ankyloblepharon, ectodermal defects and cleft palate appear to be a causally unrelated triad, but it may be possible to connect these disorders. Ectoderm, for instance, lines the fusing palatal processes. Persistence of this tissue layer along the fusion line results in the formation of small epithelial rests identifiable by microscopy (Stark, 1954). The lacrimal duct is formed from a similar 'rest' of ectoderm entrapped by the fusing nasal processes. This proliferates at about the 35 mm stage of embryonic development and the solid cord of cells canalizes by dissolution of the central core (Duke-Elder, 1964). The developing eyelids are covered with ectoderm and during the process of formation of the adult structure, both fusion and then separation of the joined parts occurs. The development of limb structure is known to be in part dependent upon a complex interaction between ectoderm and mesoderm, which, for instance, allows the development of the grooves between digits. Both syndactyly and ectrodactyly could result from a defect in this process.

The term ectodermal dysplasia is used to describe a gross inherited defect apparently confined to ectodermal tissue. However, there is evidence to suggest that other germ layers may be affected as well. For instance, Reed, Lopez & Landing (1970) described some cases in which endoderm was abnormal. We know that mesoderm and ectoderm interact in the production of limb buds, feathers, hair and other specialized structures (Wessells, 1967). A fault in mesodermal tissue's ability to organize ectoderm might produce the picture of ectodermal dysplasia. In our cases there was no evidence of involvement of another germ layer although the past history of thyroid disease in case 3 could be relevant. It is therefore not possible to incriminate one embryonic germ layer as the sole abnormality in this syndrome. But the most readily apparent features may be explained in terms of either an abnormality of ectoderm itself or a defective interaction of ectoderm and mesoderm.

CONCLUSION

This is the first time, to our knowledge, that this syndrome of ankyloblepharon, ectodermal defects and cleft lip and palate has been described. The ectodermal defects seen in the patients recorded here were very striking and generally consistent. The scalp hair was wiry and sparse or absent. The nails were abnormal or absent. The teeth were pointed and spaced, and soon worn down or lost. Hypohidrosis was demonstrated in all the affected individuals. There was a history of ankyloblepharon or ankyloblepharon filiforme adnatum in most of the affected patients and they all had a cleft palate or cleft lip/cleft palate. None of these features was individually diagnostic, but together they contributed to the unusual appearance seen in this syndrome.

ACKNOWLEDGMENTS

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REFERENCES

Bowen, P. & Armstrong, H.B. (1973) Cleft lip and palate, ectodermal dysplasia, hand and foot anomalies and oligophrenia. In: *Birth Defects Atlas and Compendium* (Ed. by Daniel Bergsma), p. 262. The National Foundation, New York.

BRILL, C.B., HSU, L.Y.F. & HIRSCHHORN, K. (1972) The syndrome of ectrodactyly, ectodermal dysplasia and cleft lip and palate: report of a family demonstrating dominant inheritance pattern. *Clinical Genetics*, **3**, 295.

COCKAYNE, E.A. (1933) *Inherited Abnormalities of the Skin and its Appendages*, pp. 218–219. Oxford University Press, London.

DUKE-ELDER, S. (1964) In: *System of Ophthalmology*. Vol. III, Part I, Chpt. VIII, pp. 240–245.

EVERETT, F.G., JUMP, E.B., SUTHERLAND, W.F., SAVARA, B.S. & SUHER, T. (1952) Anhidrotic ectodermal dysplasia with andontia—a study of 2 families. *Journal of the American Dental Association*, **44**, 173.

FRAZER, F.C. & PASHAYAN, H. (1970) The relation of face shape to susceptibility to congenital cleft lip. *Journal of Medical Genetics*, **7**, 112.

FREIRE-MAIA, N. (1970) A newly recognized genetic syndrome of tetramelic deficiencies, ectodermal dysplasia, deformed ears and other abnormalities. *American Journal of Human Genetics*, **22**, 370.

HARRIS, D.R., POLK, B.F. & WILLIS, I. (1972) Evaluating sweat gland activity with imprint techniques. *Journal of Investigative Dermatology*, **58**, 78.

HAY R.J. & WELLS, R.S. (1975) Syndrome of ankyloblepharon, ectodermal defects and cleft lip and palate. *Proceedings of the Royal Society of Medicine*, **68**, 536.

HOLLISTER, D.W., KLEIN, S.H., DE JAGER, H.J., LACHMAN, R.S. & RIMOIN, D.L. (1973) The lacrimo-auriculo-dento-digital syndrome. *Journal of Pediatrics*, **83**, 438.

HUTCHINSON, P.E. & WELLS, R.S. (1975) Hereditary hypotrichosis (Marie-Unna type). *Proceedings of the Royal Society of Medicine*, **68**, 534.

KERR, C.B., WELLS, R.S. & COOPER, K.E. (1966) Gene effect on carriers of anhidrotic ectodermal dysplasia. *Journal of Medical Genetics*, **3**, 169.

KUNO, Y. (1956) *Human Perspiration*, Chpt. 2, p. 73. Charles C. Thomas, Illinois.

LONG, J.C. & BLANDFORD, S.E. (1962) Ankyloblepharon filiforme adnatum with cleft lip and palate. *American Journal of Ophthalmology*, **53**, 126.

MICHAELIDES, A.C., HAY, R.J. & WELLS, R.S. (1975) Congenital sinuses of the lower lip. *Transactions of the St John's Hospital Dermatological Society*, **61**, 82.

RAPP, R.S. & HODGKIN, W.E. (1968) Anhidrotic ectodermal dysplasia: autosomal dominant inheritance with palate and lip anomalies. *Journal of Medical Genetics*, **5**, 269.

REED, W.B., LOPEZ, D.A. & LANDING, B. (1970) Clinical spectrum of anhidrotic ectodermal dysplasia. *Archives of Dermatology*, **102**, 134.

RUDIGER, R.A., HAASE, W. & PASSARGE, E. (1970) The association of ectrodactyly, ectodermal dysplasia and cleft lip and palate. (The E.E.C. syndrome). *American Journal of Diseases of Children*, **120**, 160.

SORSBY, A. (1970) *Ophthalmic Genetics*, Chpt. 11, p. 185. Butterworth, England.

STARK, R.B. (1954) The pathogenesis of hair lip and cleft palate. *Plastic and Reconstructive Surgery and the Transplantation Bulletin*, **13**, 20.

WESSELLS, N.K. (1967) Differentiation of epidermis and epidermal derivatives. *New England Journal of Medicine* **277**, 33.

British Journal of Dermatology (1976) **94,** 291.

The adenosine triphosphate content and lactic acid production of guinea-pig skin after mild heat damage

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SUMMARY

The depletion of adenosine triphosphate in skin after mild thermal injury is not large enough to be accounted for by loss of oxidative respiration. Measurements of lactic acid production by skin suggest that glycolysis is less sensitive to heat damage than is oxygen uptake. It is therefore likely that glycolysis makes a large contribution to the relatively high levels of ATP persisting in heat damaged skin.

The study of mild, non-coagulative heat injury must be relevant to the partly damaged areas of skin surrounding burns. The degeneration or recovery of such areas may greatly influence the healing process of burn injuries. Previous studies on the effect of 30 min exposure of skin explants to temperatures ranging from 37 to 50°C have resulted in sigmoid inhibitive curves showing that temperatures in the region of 43·5°C produce a 50% effect. The effect of heat damage on $^{35}SO_4''$ incorporation by skin was reported by Lawrence & Ricketts (1957), $^{32}PO_4'''$ incorporation by Carney, Lawrence and Ricketts (1962) and ^{14}C-proline incorporation by Carney, Lawrence & Ricketts (1965). In each of these cases the parameter measured was isotope uptake into a high molecular weight compound which required energy for its synthesis. It was therefore of interest to assess the effect of the same experimental conditions on the ATP content of skin, since this compound is the primary source of energy for most cells. As the effect of heat on overall respiration measured by oxygen uptake had already been determined by Lawrence & Ricketts (1957) with similar results to those cited above, lactic acid production was measured to estimate the contribution made by glycolysis to ATP production in these circumstances.

MATERIALS AND METHODS

The experimental system consisted of pieces of skin with equal thickness of dermis and epidermis, cut aseptically from the ears of guinea-pigs, as described by Cruickshank (1954). The slices were weighed on a torsion balance as they were cut, and collected onto sterile saline. Experiments on the effects of graded thermal damage were made by maintaining the skin samples on saline for 30-min periods at temperatures ranging from 37 to 48°C (Lawrence & Ricketts, 1957). The adenosine triphosphate (ATP) content of the skin samples was estimated as described by Carney, Hall & Ricketts (1972) using test kits supplied by Boehringer. In one series of experiments ATP measurements were made immediately after heating was discontinued. In a further series of experiments skin samples were transferred after heating to a sterile nutrient medium (Cruickshank & Lowbury, 1952) and incubated for 24 h at 37°C. At the end of the incubation period the culture medium was checked for sterility and the ATP content of the skin samples was measured.

In experiments involving lactic acid estimation, skin samples each consisted of three uniform discs cut from larger skin slices with a circular punch. These samples were incubated on 0·5 ml aliquots of medium for 24 h at 37°C. A control aliquot of medium without skin was also incubated. After incubation the skin samples were removed and lactic acid estimated in the medium after deproteinization with 2 vol. 0·6 N perchloric acid. The lactic acid content of the medium control was also estimated. The method, based on that of Hohorst (1970), uses lactic dehydrogenase to catalyse the oxidation of lactate to pyruvate according to the equation

$$\text{L-lactate} + \text{NAD}^+ \rightleftharpoons \text{pyruvate} + \text{NADH} + \text{H}^+.$$

Pyruvate is trapped by hydrazine, and the change in u.v. absorption resulting from the formation of NADH is proportional to the lactate originally present. The components of the reaction mixture were supplied by Boehringer as a test kit. Lactate concentrations were read from a standard curve.

RESULTS

The values obtained for ATP content of skin after 24 h incubation at 37°C following 30 min heating at various temperatures are presented in Table 1. The temperature producing 50% reduction of ATP content was 46·4°C. Values obtained for total lactic acid production during a 24 h incubation at 37°C,

TABLE 1. ATP content of skin after 24 h incubation at 37°C following 30 min heating on saline at various temperatures

Temperature (°C)	nmol ATP/mg F.W. of skin	s.e. mean	No. of observations	% reduction of ATP content of skin
37	1·689	0·141	3	9·8
40	1·523	0·133	3	
37	1·512	0·037	3	4·7
42	1·441	0·189	3	
37	1·253	0·075	3	
44	1·040	0·031	3	8·0
37	2·255	0·019	3	
44	2·188	0·170	3	
37	2·389	0·099	3	
46	1·668	0·059	3	37·5
37	1·726	0·227	3	
46	0·935	0·017	3	
37	0·780	0·102	3	93·6
48	0·050	0·006	3	

TABLE 2. Lactate content of medium after 24 h incubation of skin at 37°C following 30 min heating on saline at various temperatures

Temperature (°C)	nmol lactate produced/mg F.W. of skin	s.e. mean	No. of observations	Mean % inhibition of lactate production
37	555	20	3	
42	548	27	3	−7·5
37	432	38	3	
42	497	26	3	
37	710	21	3	
44	511	16	3	31·1
37	530	35	3	
44	350	8	3	
37	1064	95	3	
46	192	19	3	84·2
37	676	14	3	
46	92	5	2	
37	520			
48	−85 (after deduction of medium blank)			100
37	740			
48	−104 (after deduction of medium blank)			

following heat treatment, are presented in Table 2. The temperature producing 50% reduction in lactic acid production was 44·6°C calculated according to Gaddum (1948).

DISCUSSION

The state of the processes by which energy is produced is of obvious importance to the viability of a tissue. Skin uses both aerobic and anaerobic pathways for the production of ATP (Cruickshank, 1965) and it was of interest to attempt to determine the way in which the balance between these processes might be affected by mild heat damage.

It is apparent that the ATP content of skin is less sensitive to mild heat damage than is the ability to take up oxygen (Lawrence & Ricketts, 1957). Some comparison is possible with the data of Hammer (1973) who reported 38% loss of ATP in human skin deprived of oxygen for 30 min at 37°C. A temperature of at least 46°C for 30 min was required to produce this effect in incubated skin samples. 47·5°C was required before 38% loss of ATP was found immediately after the heating period, although measurements made at this time gave variable results. These are temperatures which cause 80–100% loss of glycolytic activity measured by lactic acid production, and absence of $^{32}PO_4'''$-labelled glycolysis intermediates as shown by paper chromatography and radio-autography (Brooks, Lawrence & Ricketts, 1959). It is possible that only about 38% of skin ATP is derived from the respiratory chain, and also possible that skin can survive without this contribution, as Medawar (1947) reported that skin maintained anaerobically for 1 week at 37°C could be grafted with satisfactory results. Carney, Hall & Ricketts (1972) reported large losses of ATP from skin thawed after storage at −196°C, conditions known to maintain skin sufficiently viable for grafting. Hershey *et al.* (1971), however, reported improved oxygen uptake and glucose utilization in heated skin supplied with exogenous ATP.

The finding that skin heated at 48°C depleted the lactic acid content of the serum medium requires further investigation. The data of Lawrence & Ricketts (1957) suggest that about 10% of the normal oxygen uptake may persist after this treatment, and it is possible that oxidation of lactate is occurring. Alternatively if lactic acid production by glycolysis is totally inhibited, some lactic acid may have been lost from the medium by diffusion into the skin.

REFERENCES

BROOKS, S.A., LAWRENCE, J.C. & RICKETTS, C.R. (1959) The phosphate esters of mammalian skin maintained on glucose and various deoxyglucoses. *Biochemical Journal*, 73, 566.

CARNEY, S.A., HALL, M. & RICKETTS, C.R. (1972) The adenosine triphosphate content of guinea-pig skin stored at −196°C. *British Journal of Dermatology*, 87, 23.

CARNEY, S.A., LAWRENCE, J.C. & RICKETTS, C.R. (1962) The incorporation of phosphate by mammalian skin in tissue culture. *Experimental Cell Research*, 27, 587.

CARNEY, S.A., LAWRENCE, J.C. & RICKETTS, C.R. (1965) The incorporation of [^{14}C] proline by mammalian skin in tissue culture. *Biochimica et Biophysica Acta*, 111, 154.

CRUICKSHANK, C.N.D. (1954) Continuous observation of the respiration of skin *in vitro*. *Experimental Cell Research*, 7, 374.

CRUICKSHANK, C.N.D. & LOWBURY, E.J.L. (1952) Effects of antibiotics on tissue cultures of human skin. *British Medical Journal*, ii, 1070.

CRUICKSHANK, C.N.D. Metabolic processes in skin. (1965) *British Journal of Dermatology*, 77, 603.

GADDUM, J.H. (1948) *Pharmacology*, 3rd edn. Oxford University Press, London.

HAMMAR, H. (1973) ATP and ADP levels and epidermal replacement rate in normal human skin and in some papulosquamous diseases of the skin. *Acta dermato-venereologica*, 53, 251.

HERSHEY, F.B., IM, M.J.C., KIM, D.H. & HOOPER, J.E. (1971) Effect of ATP on glucose metabolism of thermally injured skin *in vitro*. *Journal of Trauma*, 11, 931.

HOHORST, H.J. (1970) In: *Methods of Enzymatic Analysis* (Ed. by H.O.Bergmeyer) Vol. 11, p. 1425. Verlag Chemie, Weinheim.

LAWRENCE, J.C. & RICKETTS, C.R. (1957) The metabolic uptake of sulphate ions by skin. *Experimental Cell Research*, 12, 633.

MEDAWAR, P.B. (1947) The behaviour of mammalian skin epithelium under strictly anaerobic conditions. *Quarterly Journal of Microscopical Science*, 88, 27.

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The succinic dehydrogenase and cytochrome c oxidase activities of guinea-pig skin after mild heat damage

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SUMMARY

Succinic dehydrogenase and cytochrome c oxidase activities of guinea-pig ear skin were found to be less sensitive than oxygen uptake to mild heat damage. It therefore appears that the failure of oxygen uptake following thermal injury is not directly due to inactivation of the enzymes of the respiratory chain.

No single parameter can be regarded as an infallible index of the viability of a tissue, but the experience of many workers has shown that ability to take up oxygen is a very useful guide (Cruickshank, Trotter and Cooper, 1957; Lawrence, 1968, 1972). Oxygen uptake has also been used to quantify mild thermal damage to skin (Lawrence & Ricketts, 1957), 30% loss of oxidative respiration representing a threshold above which the tissue suffers irreversible damage (Lawrence, 1959). It was therefore of interest to study the behaviour of enzymes concerned with oxygen uptake under conditions of mild heat damage. Succinic dehydrogenase and cytochrome c oxidase are key enzymes in this process, the succinic dehydrogenase complex being a component of the Krebs' cycle and the respiratory chain, and cytochrome c oxidase providing the chain with its final link with molecular oxygen by transferring electrons to oxygen from reduced cytochrome c.

MATERIALS AND METHODS

The experimental system consisted of pieces of skin having equal thickness of dermis and epidermis cut aseptically from the ears of guinea-pigs as described by Cruickshank (1954). The skin slices were weighed on a torsion balance as cut, and collected onto sterile physiological saline solution. Skin samples were heated on saline for 30 min at temperatures ranging from 37 to 48°C. After heating samples were used for the estimation of succinic dehydrogenase activity, or were transferred to a nutrient medium (Cruickshank & Lowbury, 1952) and incubated at 37°C for 24 h. At the end of the incubation period the sterility of the medium was checked, and the skin samples used for the estimation of succinic dehydrogenase or cytochrome c oxidase activity.

TABLE 1. Rate of formazan production by skin incubated at 37° C on Krebs Ringer phosphate medium containing 0·074 mol/l sodium succinate and varying concentrations of 2,3,5 triphenyltetrazolium chloride

2,3,5, triphenyl tetrazolium chloride concn. (mol/l)	nmol formazan produced/min /mg F.W. of skin	s.e. mean	No. of observations
0·004	0·212	0·010	4
0·005	0·221	0·015	20
0·010	0·263	0·015	20
0·020	0·265	0·015	20
0·040	0·288	0·017	24
0·080	0·284	0·009	4

The method used for the estimation of succinic dehydrogenase activity was based on that described by Hershey, Cruickshank & Mullins (1958). An important modification consisted of increasing the concentration of 2, 3, 5 triphenyl tetrazolium chloride to 0·04 mol/l. Table 1 shows that maximum reduction per unit weight of skin is not achieved under these experimental conditions unless this concentration of tetrazolium salt is used. Entry of the compound into undamaged cells probably reaches a limiting rate at this concentration. The sensitivity of the method is reduced unless undamaged specimens demonstrate maximum enzyme activity. Results were calculated as described by Altman (1969).

Cytochrome c oxidase activity was assayed by a method essentially that of Smith (1956). Pooled skin samples averaging 80 mg in weight were homogenized in 1·5 ml 0·1 M phosphate buffer pH 7, with cooling. A Polytron PCU-2 homogenizer with a PT-10 head was used for the purpose. Homogenates were centrifuged at 2000 rev/min for 5 min and the supernatants immediately used for the estimation of cytochrome c oxidase activity. Cytochrome c (Boehringer) was prepared as a 90 μmol/l solution in 0·01 mol/l phosphate buffer pH 7, and reduced by gassing with hydrogen for 1 h in the presence of a palladium–asbestos catalyst (B.D.H.) which was subsequently filtered off. The reaction mixture in a 1 cm cuvette, consisted of 2·0 ml 0·1 mol/l phosphate buffer pH 7, 0·5 ml reduced cytochrome c solution (90 μmol/l) and 0·5 ml enzyme solution. The course of the reaction was followed using a SP.500 (Unicam) spectrophotometer at a wavelength of 550 nm, and plotted by a Unicam AR 45 recorder at a chart speed of 20 s/cm. Recording was continued for 3 min. 0·01 ml saturated potassium ferricyanide solution was added to the cuvette, the reaction mixture stirred, and optical density of the fully oxidized cytochrome c recorded. Initial rate of reaction was determined from the chart. A factor relating change in optical density to nmol cytochrome c oxidized was determined experimentally for the conditions being used. Results were expressed in terms of nmol cytochrome c oxidized/unit time/unit fresh weight of tissue.

RESULTS

Values obtained for succinic dehydrogenase activity of guinea-pig ear skin immediately after heat treatment are recorded in Table 2. Table 3 presents values obtained from skin samples incubated for 24 h at 37°C following mild heat damage. It is apparent that injury is progressive after the skin has been returned to physiological temperatures. The temperature producing 50% effect when applied for 30 min was 47·6°C for unincubated samples, and 45·3°C for samples after 24 h incubation at 37°C.

The apparent increase in enzyme activity after treatment at 42°C may represent an increase in cell permeability, allowing more substrate to enter but not damaging the enzyme system.

Values obtained for cytochrome c oxidase activity of heated guinea-pig skin after incubation are given in Table 4. The damaging temperature required to produce 50% effect was 45·9°C. Lawrence & Ricketts (1957) reported that a temperature of 43·5°C for 30 min resulted in 50% loss of oxygen uptake by skin samples incubated for 24 h at 37°C.

TABLE 2. Succinic dehydrogenase activity of skin immediately after 30 min heating at various temperatures

Temperature (°C)	nmol formazan /min/mg F.W. of skin	s.e. mean	No. of observations	% inhibition
37	0·303	0·013	3	
40	0·333	0·010	3	−9·9
37	0·337	0·028	4	
42	0·395	0·024	4	−17·2
37	0·428	0·020	4	
44	0·355	0·010	4	17·1
37	0·346	0·004	4	
46	0·270	0·008	3	22·0
37	0·287	0·012	4	
48	0·124	0·004	4	56·8

TABLE 3. Succinic dehydrogenase activity of skin after 24 h incubation at 37° C, following 30 min heating at various temperatures

Temperature (°C)	nmol formazan /min/mg F.W. of skin	s.e. mean	No. of observations	% inhibition
37	0·561	0·069	4	
40	0·532	0·007	3	5·2
37	0·326	0·023	3	
42	0·384	0·026	3	−17·8
37	0·548	0·019	4	
44	0·409	0·034	3	25·4
37	0·620	0·039	3	
46	0·236	0·013	4	62·0
37	0·306	0·008	4	
48	0·019	0·002	4	93·8

DISCUSSION

The ability of skin to take up oxygen has proved to be a useful index of the viability of the tissue in determining the effect of potentially toxic agents (Lawrence, 1968), in connection with the problems of storage of skin required for grafting (Lawrence, 1972), and in assessing heat damage (Lawrence & Ricketts, 1957). It was therefore of particular interest to find that two important oxidative enzymes

TABLE 4. Cytochrome c oxidase activity of skin after 24 h incubation at 37° C, following heating at various temperatures

Temperature (°C)	nmol cytochrome c oxidized/min /mg F.W. of skin	s.e. mean	No. of observations	% inhibition
37	0·0213	0·0009	3	
42	0·0210	0·0001	3	1·4
37	0·0107	0·0021	5	
45·5	0·0065	0·0002	5	39·3
37	0·0131	0·0025	4	
48	0·0018	0·0008	4	86·3
37	0·0266	0·0009	3	
50	0·0003		3	98·9

were considerably more resistant to mild thermal injury than the overall process of oxygen uptake. This suggests that when glucose is provided as the energy source oxygen uptake in heated skin diminishes initially as a result of the failure of a reaction or reactions prior to the respiratory chain. Carney, Hall & Ricketts (1976) found that glycolysis, measured by lactic acid production, was also more resistant than oxygen uptake to mild thermal injury. It is therefore tempting to suggest that the most heat sensitive step in the energy producing processes of skin lies in the Krebs' cycle. Hershey *et al.* (1965), in a study of the effects of heat on enzymes of skin homogenates, reported that fumarase is particularly heat labile, activity being reduced by 70% after 5 min heating at 45°C. However, although there is now little doubt that a functional Krebs' cycle exists in skin (Cruickshank, 1964) its relative importance is not established. The finding of Cruickshank *et al.* (1957) that succinate as substrate could not maintain oxygen uptake of skin at the level produced by glucose, whereas lactate and pyruvate maintained respiration at higher levels, emphasizes the central importance of acetyl coenzyme A in the oxidative respiration of skin. This compound is required for the operation of the Krebs' cycle and also for the synthesis of fatty acids, some of which are known to support skin respiration (Barron, Meyer & Miller, 1948; Anderson *et al.*, 1958). Lipid provides 70% of the endogenous substrate of skin when an exogenous substrate is not available (Cruickshank, Trotter & Cooper, 1962), and it is possible that fatty acids are produced and oxidized preferentially even when a carbohydrate substrate is supplied. It is hoped that work at present in progress will help to answer these questions.

REFERENCES

ALTMAN, F.P. (1969) The use of eight different tetrazolium salts for a quantitative study of pentose shunt dehydrogenation. *Histochemie*, 19, 362.

BARRON, E.S.G., MEYER, J. & MILLER, Z.B. (1948) Metabolism of skin. Effect of vesicant agents. *Journal of Investigative Dermatology*, 11, 97.

CARNEY, S.A., HALL, M. & RICKETTS, C.R. (1976) The adenosine triphosphate content and lactic acid production of guinea-pig skin after mild heat damage. *British Journal of Dermatology*, 94, 291.

CRUICKSHANK, C.N.D. (1954) Continuous observation of the respiration of skin *in vitro*. *Experimental Cell Research*, 7, 374.

CRUICKSHANK, C.N.D. (1964) The metabolism of mammalian epidermis *in vitro*. *Symposium of the Zoological Society of London*, 12, 25.

CRUICKSHANK, C.N.D. & LOWBURY, E.J.L. (1952) Effects of antibiotics on tissue cultures of human skin. *British medical Journal*, ii, 1070.

Cruickshank, C.N.D., Trotter, M.D. & Cooper, J.R. (1957) Studies on the carbohydrate metabolism of skin. *Biochemical Journal*, **66**, 285.

Cruickshank, C.N.D., Trotter, M.D. & Cooper, J.R. (1962) A study of available substrates for the endogenous respiration of skin *in vitro*. *Journal of Investigative Dermatology*, **39**, 175.

Hershey, F.B., Cruickshank, C.N.D. & Mullins, L.I. (1958) The quantitative reduction of 2,3,5-triphenyl tetrazolium chloride by skin *in vitro*. *Journal of Histochemistry and Cytochemistry*, **6**, 191.

Hershey, F.B., Lewis, C., Johnston, G., Murphy, J., Mullins, L. & Schiff, T. (1965) Effects of heat on pyridine nucleotide dehydrogenases, aldolase and fumarase of human epidermis. *Journal of Investigative Dermatology*, **44**, 93.

Lawrence, J.C. (1959) The comparative toxicity of antibiotics to skin. *British Journal of Pharmacology and Chemotherapy*, **14**, 168.

Lawrence, J.C. (1968) The effect of silver nitrate, sulfamylon and other antibacterial agents on the metabolism of mammalian skin. *Excerpta Medica International Congress Series*, **190**, 234.

Lawrence, J.C. (1972) Storage and skin metabolism. *British Journal of Plastic Surgery*, **25**, 440.

Lawrence, J.C. & Ricketts, C.R. (1957) The metabolic uptake of sulphate ions by skin. *Experimental Cell Research*, **12**, 633.

Smith, L. (1956) *Methods of Biochemical Analysis*, Vol. II (Ed. by D. Glick), p. 427. Interscience Publishers, New York and London.

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Enzyme activity in human scars, hypertrophic scars and keloids

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SUMMARY

Biopsies from non-hypertrophic and hypertrophic scars and from normal skin have been studied histochemically for activities of nicotinamide adenine dinucleotide diaphorase, lactate dehydrogenase, acid phosphatase, β-D glucuronidase and alkaline phosphatase.

The activities of all enzymes studied except alkaline phosphatase were found to be increased in hypertrophic scars as compared with non-hypertrophic scars and normal skin.

The early process of healing of the excised wound has been extensively studied using histochemical techniques (Fatteh, 1965; RaeKallio, 1970). The mechanisms which are responsible for long-term changes in scar tissue have also been investigated but still remain obscure (Madden & Peacock, 1971).

Increases in activity of some enzymes participating in the Embden-Meyerhof glycolytic pathway, the tricarboxylic acid cycle and the pentose phosphate shunt have already been described (Hoopes, Su & Im, 1971). Using fluorometric assay techniques, they found increases in activity of several enzymes including glucose-6-phosphate dehyrogenase, hexokinase and pyruvate kinase in hypertrophic and keloid scars compared with normal skin.

Craig (1973) studied the collagenase activity of mature scars, hypertrophic and keloid scars. Compared with normal skin, all of these scars showed increased collagenase activity. Hypertrophic and keloid scars also showed greater collagenase activity than non-hypertrophic scars.

The present study was designed to investigate by histochemical techniques the enzymic processes which result in the formation of the hypertrophic or keloid scars.

MATERIALS AND METHODS

The study was carried out by taking fifty-five biopsies from hypertrophic scars of patients presenting for treatment, and from twenty-four non-hypertrophic scars presenting for scar revision. Normal skin was obtained from twelve patients undergoing abdominal and breast reductions and prominent

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TABLE I. Methods, pH of the media and incubating temperature used for the demonstration of enzyme activity

Enzyme	Author	Substrate	pH of Incubation media	Incubation time (min)	Temperature of incubation (°C)
N.A.D. diaphorase	Nachlas *et al.* (1958)	Reduced N.A.D.	7·4	60	Room temp.
Lactate dehydrogenase	Hess *et al.* (1958)	Sodium lactate	8·0	20	37
β-D-Glucuronidase	Hayashi *et al.* (1964)	Napthol AS-B1 glucuronide	5·1	60	37
Acid phosphatase	Barka & Anderson (1963)	Sodium α-naphthyl phosphate	5·5	20	37
Alkaline (i) phosphatase	Menten *et al.* (1944)	Sodium α-naphthyl phosphate	8·0	10	Room temp.
(ii)	Burstone (1958)	Napthol AS-CL phosphate	8·3	120	37

ear corrections. A 5 mm cone was biopsied with a Roxburgh skin trephine from the centre of the scars.

The skin was fixed in formol calcium at 4°C for ½ h and then stored in Holt's gum sucrose at 4°C until sectioned. Cryostat sections were cut at 15 μm, mounted direct from the knife on to the slides, and then air dried for 15 min. The method of demonstration, pH and time of incubation of the enzymes are shown in Table 1.

The specificity of the enzyme reactions was confirmed by:

(a) Incubation without substrate; (b) incubation in the presence of specific inhibitors (Table 2).

Representative cryostat sections were taken and further fixed in formol alcohol, prior to staining with haematoxylin and eosin for histological study.

The enzyme activity of the non-hypertrophic and hypertrophic scars was compared with the activity in normal skin and then categorized as follows:

N, activity similar to normal skin; N+1, a moderate increase in activity as compared to normal skin; N+2, a marked increase in activity as compared to normal skin; N+3, a very intense activity as compared to normal skin.

TABLE 2. The specific inhibitors of enzyme activity of human skin

Enzyme	Inhibitor	Concentration in incubation medium
N.A.D. diaphorase	p-hydroxymercuribenzoate	1 mM
Lactate dehydrogenase	p-chloromercuribenzoate	1 mM
β-D-glucuronidase	D. gluco-saccharo- 1:4 lactone	$2{\cdot}5 \times 10^{-6}$ M
Acid phosphatase	Sodium fluoride	0·1 M
Alkaline phosphatase	L-cysteine	10^{-3} M

RESULTS

The histological appearances of the non-hypertrophic and hypertrophic scars did not materially differ from those previously described (Cosman *et al.*, 1961; Mancini & Quaife, 1962).

In most hypertrophic scars, there was thinning of the epidermis with flattening of the dermal papillae. In some hypertrophic scars, hyaline collagen fibres were present, but any differentiation between keloid and hypertrophic scars by histological criteria was inconclusive.

Nicotinamide adenine dinucleotide (N.A.D.) diaphorase

In the dermis of normal skin there was some N.A.D. diaphorase in the fibroblasts, but more in skin appendages, sebaceous and sweat glands. In the epidermis highest activity was found in the basal layers, only moderate activity being present in the strata lucidum and granulosum. There was no activity in the surface layers of the stratum corneum. Incubation without substrate or in the presence of p-hydroxymercuribenzoate caused complete loss of activity in all cells except the hair follicles.

In the non-hypertrophic scars, there was a moderate activity in the dermal fibroblasts, which were frequently distributed in tracts. The appearance of the hypertrophic scars was quite different; there was a very marked increase in activity of the enzyme throughout the dermal fibroblasts and in many instances a distribution into two layers, a superficial layer of less activity (N+2) and a deeper layer with a more intense activity (N+3) (Kemble & Brown, 1974).

Lactate dehydrogenase

Normal scar had a moderately increased lactate dehydrogenase activity (N+1) compared with normal skin. Activity in hypertrophic scar was greatly increased in both dermis (N+3) and epidermis (N+2).

Incubation without substrate or N.A.D. caused a marked reduction in activity. Incubation with p-chloromercuribenzoate caused complete inhibition.

Acid phosphatase

Acid phosphatase activity was present in the stratum granulosum, in skin appendages and in fibroblasts of normal skin, and in non-hypertrophic scars activity was similar (N). In hypertrophic scar there was a moderate increase in activity, more so in the deeper layers (N+2) than in the more superficial layers of the dermis (N+1).

β-D glucuronidase

In normal skin, the enzyme was active in all layers of the epidermis except stratum corneum, in sweat glands, hair follicles and a few dermal fibroblasts. In non-hypertrophic scar activity was similar. In hypertrophic scar, however, there was a moderate increase in lysosomal and extralysosomal activity (N+2).

Alkaline phosphatase

Alkaline phosphatase was active in hair follicles, capillary endothelium and in dermal papillae of normal skin. This activity was completely inhibited by addition of 10^{-3} M L-cysteine. The only site of increased activity in hypertrophic scar compared to normal skin and non-hypertrophic scar was in capillary endothelium; activity was otherwise similar in all three types of tissue.

DISCUSSION

The functions of many enzymes in skin are at present speculative. The very marked increase in activity of N.A.D. diaphorase in hypertrophic scar, however, is indicative of a potential for increased energy production in mitochondria, and more specifically from the tricarboxylic cycle in the process of production of adenosine triphosphate. Acid phosphatase is concerned with connective tissue repair (Raekallio, 1970) so that the finding of a raised activity in hypertrophic scar may indicate the abnormal metabolic pathway which results in hypertrophic scar formation.

It is generally agreed that β-D glucuronidase, present mainly in lysosomes, is basically a lytic enzyme (Monro, Canedo & Soberon, 1965) although it may have some function in synthetic processes (Raekallio, 1970). The increased β-D glucuronidase activity which has been demonstrated may in part be evidence of increased mucopolysaccharide degradation. There is also considerable experimental evidence that β-D glucuronidase is concerned in the mechanism of phagocytosis (Ballantyne, 1970; Ballantyne & Guillon, 1971; Saito & Suter, 1965). This coupled with the demonstration that steroid hormones enhance β-D glucuronidase activity (Ballantyne & Guillon, 1971; Levy & Conchie, 1966) may explain the beneficial effect that corticosteroid therapy has on hypertrophic scar (Bartalos & Gyorkey, 1963; Im & Hoopes, 1972).

Alkaline phosphatase possesses a hydroxyl group which is responsible for the release of inorganic phosphate necessary for cell membrane transport. Little or no alteration in activity was noted in either type of scar compared with normal skin, so it is probable that scar metabolism does not include that part of cell membrane transport involving alkaline phosphatase pathways.

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REFERENCES

BALLANTYNE, B. (1970) Functional correlates of 17 β-oestradiol, β-glucuronidase and phagocytosis. *Journal of Physiology*, **210**, 72P.

BALLANTYNE, B. & GUILLON, P.J. (1971) The influence of 17 β-oestradiol colloidal particles on β-glucuronidase activity in mammalian lymph nodes. *Microbiology*, **3**, 23.

BARKA, T. & ANDERSON, P.J. (1963) *Histochemistry*. Harper and Row, New York.

BARTALOS, M. & GYORKEY, F. (1963) *Journal of American Geriatrics Society*, **11**, 21.

BURSTONE, M.S. (1958) Histochemical demonstration of acid phosphatase with napthol A.S. phosphates. *Journal of the National Cancer Institute*, **21**, 523.

COSMAN, B., CRIKELAIR, G.F., JU, D.M.C., GAVLIN, J.C. & LATTES, R. (1961) Surgical treatment of keloids. *Plastic and Reconstructive Surgery, and The Transplantation Bulletin*, **27**, 335.

CRAIG, P. (1973) Collagenase activity in cutaneous scars. *The Hand*, **5**, 239.

FATTEH, A. (1965) Enzyme changes in the early phases of wound healing. *Journal of the J.J. Group of Hospitals and Grant Medical College*, **10**, 197.

HAYASHI, M., NAKAJIMA, Y. & FISHMAN, W.H. (1964) Cytologic demonstration of β-glucuronidase employing napthol AS-BI glucuronide and hexazonium pararosaniline. *Journal of Histochemistry and Cytochemistry*, **12**, 293.

HESS, R., SCARPELLI, D.G. & PEARSE, A.G.E. (1958) Cytochemical localisation of pyridine nucleotide-lined dehydrogenase. *Nature*, **181**, 1531.

HOOPES, J.E., SU, C. & IM, M.J.C. (1971) Enzyme activities in hypertrophic scars. *Plastic and Reconstructive Surgery and the Transplantation Bulletin*, **47**, 132.

IM, M.J.C. & HOOPES, J.E. (1972) Enzyme activities in regenerating epithelium. *Journal of Surgical Research*, **12**, 406.

KEMBLE, J.V.H. & BROWN, R.F.R. (1974) Histochemistry of hypertrophic scars and keloids. *Proceedings of the Royal Society of Medicine*, **67**, 257.

LEVVY, K. & CONCHIE, J. (1966) β-glucuronidase and the hydrolysis of Glucuronides. In: *Glucuronic Acid* (Ed. by G.T.Dutton) Academic Press, New York.

MADDEN, J.W. & PEACOCK, E.E. (1971) Studies on the biology of Collagen. *Annals of Surgery*, **174**, 511.

MANCINI, R.E. & QUAIFE, J.V. (1962) Histogenesis of experimentally produced keloids. *Journal of Investigative Dermatology*, **38**, 143.

MENTEN, M.L. JUNGE, J. & GREEN, M.H. (1944) A coupling histochemical azo-dye test for alkaline phosphatase in the kidney. *Journal of Biological Chemistry*, **153**, 471.

MONRO, J., CANEDO, L. & SOBERON, G. (1965) On the metabolic role of β-glucuronidase. *Biochemica et Biophysica Acta*, **101**, 137.

NACHLAS, M.M., WALKER, D.G. & SELIGMAN, A.M. (1958) A histochemical method for demonstration of diphosphopyridine nucleotide diaphorase. *Journal of Biophysical and Biochemical Cytology*, **4**, 29.

RAEKALLIO, J. (1970) *Enzyme Histochemistry of Wound Healing*. Fischer, Stuttgart.

SAITO, K. & SUTER, E. (1965) Lysosomal acid hydrolases in mice injected with B.C.G. *Journal of Experimental Medicine*, **121**, 727.

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Pathogenesis associated with hair follicle mites (*Demodex* spp.) in Australian Aborigines

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SUMMARY

Demodex folliculorum and *D. brevis* are found in different habitats in the skin: the former in the hair follicles, the latter in the sebaceous glands. Both seem minor pathogens—merely harvesting the cells of their respective habitats. *D. folliculorum* was found aggregated (all stages) and plugging the follicular orifices; *D. brevis*, on the other hand, was usually solitary. In one case marked degeneration of the sebaceous gland, with leukocyte invasion, was associated with *D. brevis*.

No correlations were discovered between either of these species and the gross condition of the skin. Positive samples were obtained from both mainland and island populations of Aborigines. Incidence values obtained were 66·6% for males and 80% for females; in the latter, the sample was small (5) and their ages over 34 years.

The discovery of *Demodex brevis* and *D. folliculorum* in Australian Aborigines (Nutting & Green, 1974) from material expressed from the nasolabial folds has led to an examination of the possible pathogenicity of these mites. Such a study, plus some further information on hair follicle mite incidence, forms the substance of this report.

MATERIALS AND METHODS

Small necropsy segments of skin from the nasolabial folds of three Aborigines (2 males, 1 female) were fixed in buffered formalin. Two similar segments of skin (1 from a male, 1 from a female) from the nape of the neck were likewise prepared. The specimens were processed by the paraffin–piccolyte method (Cloney, 1961—minus beeswax according to Potswald, personal communication) and cut at 8–10 μm. Sections were stained using haematoxylin–eosin, Lillie's ferrous iron, and Verhoeff's elastin procedure (Humason, 1962). Specimens in sebum and in sections were determined to species using the characteristics recorded by Desch & Nutting (1972).

Sebum, obtained as previously described (Nutting & Green, 1974), was preserved in 70% alcohol, centrifuged or sedimented, placed on slides, blotted gently, and mounted in Hoyer's medium (Baker & Wharton, 1952).

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Specimens were obtained in the Northern Territory from Aborigines living at Maningrida (11 males), Bamyili (4 males, 1 female), Milingimbi Island (3 males, 2 females) and Elcho Island (2 females) during the month of November 1973.

All sections and sebum slides were examined using light and phase contrast microscopy.

Descriptive notes on the clinical skin condition or diseases associated with the skin of the nasolabial folds were made on sixteen (15 males, 1 female) of the individuals sampled for sebum at Maningrida and Bamyili.

RESULTS

The three skin sections taken after death from the nasolabial fold were positive for both *Demodex brevis* and *D. folliculorum*. The former was found in the sebaceous glands (Fig. 1), the latter in the hair follicles above, or at the level of, the sebaceous gland ducts (Figs 2 and 5). For *D. brevis* it seemed apparent that a limited number of cells of the sebaceous gland had been destroyed. In one instance (Fig. 3) some invasion by leukocytes and degenerative changes in the gland were found adjacent to the mite. In several cases two specimens were found (adult female and nymph or larva) in the same sebaceous lobule (Fig. 4); in one case, two adjacent lobules of a single sebaceous gland each held a specimen (Fig. 1).

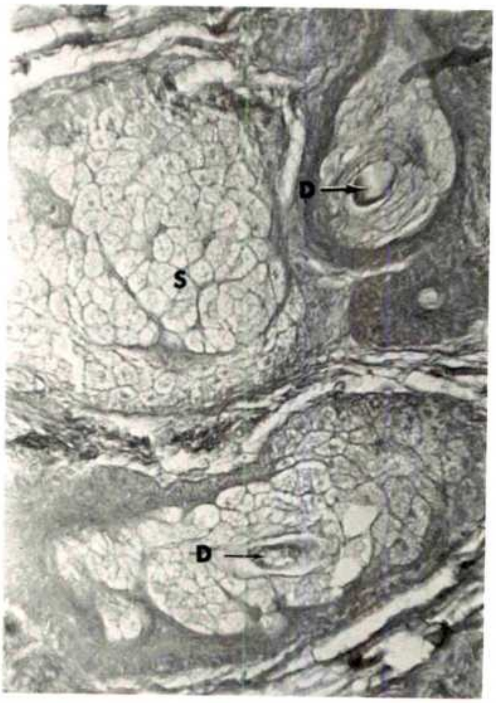

FIGURE 1. Photomicrograph of section from skin of the nasolabial fold showing *Demodex brevis* (D) in adjacent lobules of a sebaceous gland (S). (Magnification × 160.)

Invasions of *D. folliculorum* in a single hair follicle were often so heavy that the bodies of the mites plugged the follicular orifice (Fig. 5). All stages were found between the sebaceous duct and the follicle orifice; the opisthosomae of adults often protruded beyond the orifice opening. Mouthparts of adults, nymphs, protonymphs and larvae were closely apposed to the follicular epithelium. A small

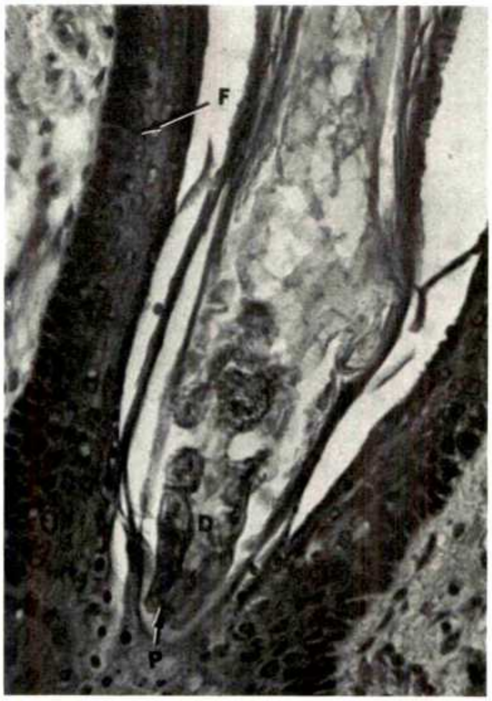

FIGURE 2. *Demodex folliculorum* (D) in hair follicle (F). Note mouthparts (P) closely apposed to cells of follicular epithelium (arrow from F). (Magnification × 360.)

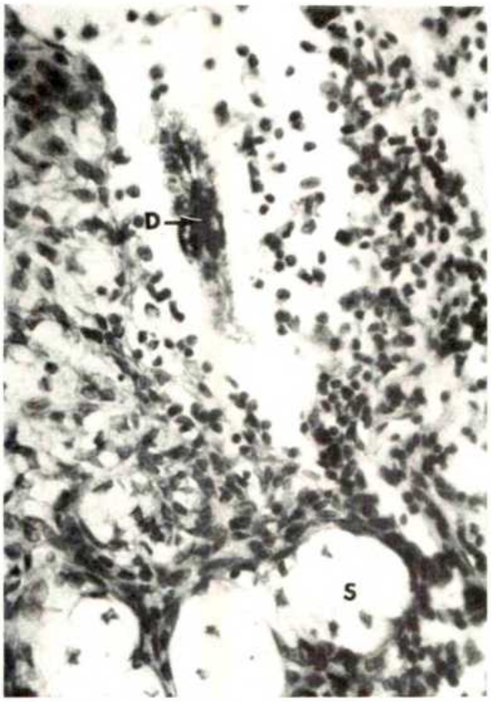

FIGURE 3. *Demodex brevis* (D) surrounded by host leukocytes in deranged lobule of sebaceous gland (S). (Magnification × 360.)

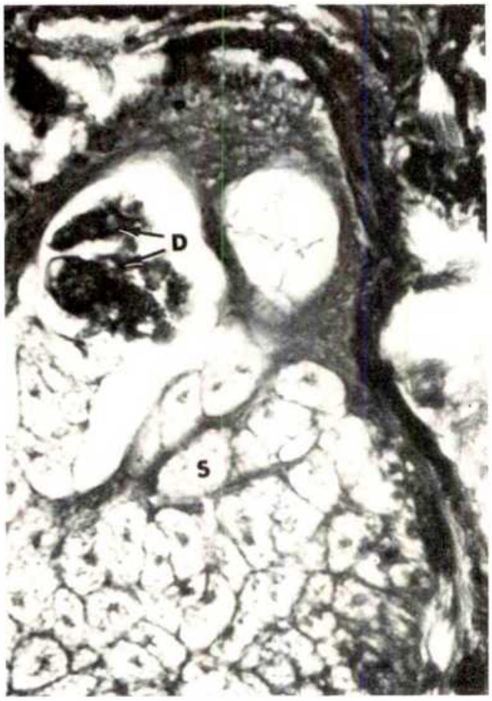

FIGURE 4. Two specimens of *D. brevis* (D) in sebaceous gland (S). (Magnification × 360.)

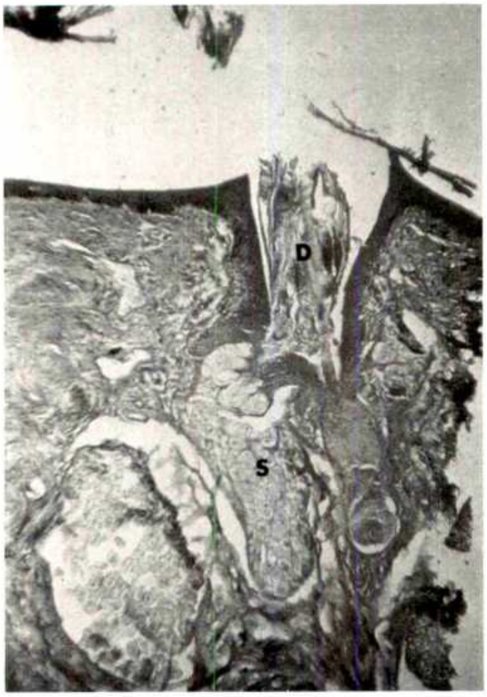

FIGURE 5. Plug of *D. folliculorum* (D) and keratin in orifice of hair follicle above sebaceous gland (S). (Magnification × 80.)

amount of cellular undercutting (Fig. 2) was found near the mouthparts indicating cell destruction.

Demodicids were not found in any sections taken from the nape.

Records of incidence are given in Table 1. Demodicids were found in both mainland (Maningrida and Bamyili, NT) and island (Milingimbi and Elcho, NT) Aboriginal populations. The incidence for females was very high (80%); the sample was, however, small (n = 5) and the ages high (35–51 years). A specimen taken from a male, approximately 21 years of age, was positive. The youngest female in whom a positive result was found was approximately 35 years.

TABLE 1. Sebum specimens positive for either (or both) species of demodicid. All samples obtained during November 1973

Place	Age*	No. positive
Maningrida, NT		
(11 males)	24–60	8
Bamyili, NT		
(4 males)	21–53	3
(1 female)	42	1
Milingimbi Island, NT		
(3 males)	25–48	1
(2 females)	35–51	2
Elcho Island, NT		
(2 females)	38–47	1
Totals		
(18 males)		12 males (66·6%)
(5 females)		4 females (80%)
23		16 (69·5%)

* All ages ±5 years.

Specimens of mites were recovered from individuals with (1) normal skin (3 of 5), (2) cases of acne, and (3) skin with patulous pilo-sebaceous orifices. No correlation was found between either the quantity of sebum expressed or the skin condition and the species of mites obtained.

DISCUSSION

Demodex brevis and *D. folliculorum* are, as reported by Desch & Nutting (1972) for Caucasians, ecologically isolated. Furthermore, *D. brevis* is solitary whereas *D. folliculorum* is 'colonial'.

The one instance of marked host cellular response (*D. brevis*, Fig. 3) seems typical for the early stages of an inflammatory reaction which has been reported, associated with *Demodex* spp. in man by Bergstad (1925).

Recently, Grosshans, Dremer & Maleville (1974) have studied pathogenesis due to *D. folliculorum* and *D. brevis* in Caucasians. As did Bergstad (1925) they found a granulomatous condition associated with *D. folliculorum*. In their account they seem to have accepted uncritically assertions made before 1972 which grouped *D. folliculorum* and *D. brevis* for taxonomy, length of life cycle and pathogenesis. They did refer to Desch & Nutting (1972) but recorded the two as sub-species (Unterarten). As has been suggested earlier (Beerman & Stokes, 1934), any causal connection between rosacea and demodicids is still subject to debate. One possible explanation is that rosacea initiated the early stages of a granulomatous condition.

We believe that the high incidence of infestation recorded for females is related to the age of the individuals sampled (the older of either sex probably harboring larger populations of mites) (Nutting & Green, 1974). It should be noted, however, that all samples in this report were obtained in the month of November. Specimens in our 1974 report were collected in February or March. No attempt has been made to date to see whether there is a seasonal fluctuation in the numbers of demodicids of man despite the fact that such has been noted for *Demodex bovis* of cattle by Poliakov (1957) and for *Demodex* spp. of the horse (Bennison, 1943).

The incidence in males (66·6%) is nearly identical to our previous finding of 66% (Nutting & Green, 1974). In all cases, we must remember the restrictions placed on the precision of these values by the very small size, and difficulties of recovery, of these mites. Only extensive sampling replications for each individual would reveal the absolute incidence which could well be 100% (Fuss, 1933).

It should also be noted that the absence of any correlation of demodicid infestation with skin condition is only suggestive; so far records of skin condition *vis-à-vis* demodicids of Aborigines have been obtained on only twenty-nine individuals (20 males: 9 females) with an incidence of 41%. Only by steady accumulation of a large sample of observations can we be assured of either a positive or a negative correlation between skin condition and either demodicid species.

ACKNOWLEDGMENTS

We would like to thank Margaret Nutting for her technical help. Dr Clifford E.Desch provided some assistance with the photography. We also thank the Aborigines who willingly co-operated to provide the specimens for our study. Without their agreement this paper could not have been written.

REFERENCES

BAKER, E.W. & WHARTON, G.W. (1952) *An Introduction to Acarology*, p. 10. Macmillan, New York.

BEERMAN, H. & STOKES, J.H. (1934) Rosacea complex and *Demodex folliculorum*. *Archives of Dermatology and Syphilology*, **29**, 874.

BENNISON, J. (1943) Demodicosis of horses with particular reference to equine members of the genus *Demodex*. *Journal of the Royal Army Veterinary Corps (Great Britain)*, **14**, 34 and 66.

BERGSTAD, E. (1925) Fall von Hautveränderung Hervorgerufen durch *Demodex folliculorum*. *Acta dermato-venereologica*, **6**, 329.

CLONEY, R.A. (1961) Observations on the mechanisms of tail resorption in ascidians. *American Zoologist*, **1**, 67.

DESCH, C.E. & NUTTING, W.B. (1972) *Demodex folliculorum* (Simon) and *D. brevis* akbulatova of man: redescription and re-evaluation. *Journal of Parasitology*, **58**, 169.

GROSSHANS, E.M., DREMER, M. & MALEVILLE, J. (1974) *Demodex folliculorum* und die Histogenese der granulomatosen Rosacea. *Hautarzt*, **25**, 166.

FUSS, F. (1933) Parasitic life of *Demodex folliculorum hominis*. *Annales de dermatologie et de syphiligraphie*, **4**, 1053.

HUMASON, G.L. (1962) *Animal Tissue Techniques*, pp. 166, 242. W.H. Freeman, San Francisco.

NUTTING, W.B. & GREEN, A.C. (1974) Hair follicle mites (Acari: Demodicidae) from Australian Aborigines. *Australasian Journal of Dermatology*, **15**, 10.

POLIAKOV, D. (1957) To the problem of demodicosis epizootology, its clinical characteristics and diagnosis in large horned cattle. (Russian text). *Trudy Vsesoyuznogo nauchno-issledovatel'skogo instituta veterinarnoi sanitarii i éktoparazitologii*, **11**, 173.

POTSWALD, H.E. Personal communication. Department of Zoology, University of Massachusetts, Amherst, Mass. U.S.A.

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Pharmacology and Treatment

The unwanted effects of clindamycin in acne

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SUMMARY

A review of seventy matched acne patients on clindamycin and seventy-two on tetracycline shows that nine patients on clindamycin had diarrhoea whereas no patients on tetracycline had any side effect; no frank pseudomembranous colitis was seen. Although we heed the side effects of clindamycin, it should not be withheld from patients with severe and resistant acne.

Clindamycin has been shown to be an effective alternative to tetracycline in patients with moderate and severe acne (Cunliffe, Cotterill & Williamson, 1972; Cunliffe & Cotterill, 1973). This is especially true in those patients whose *Corynebacterium acnes* are resistant to tetracycline. Since 1972 we have used oral clindamycin in the long-term treatment of this type of acne; because of the recent reports (Scott, Nicholson & Kerr, 1973; Tedesco, Barton & Alpers, 1974) of pseudomembranous colitis we assessed the side effects of clindamycin in our patients with matched patients taking tetracycline.

PATIENTS AND METHODS

We investigated 142 patients all of whom had had acne for 4–7 years. Seventy patients (21 males and 49 females) aged 14–51 years had taken clindamycin 150 mg twice daily for 3–8 months (mean $7{\cdot}7 \pm 0{\cdot}8$ s.e.m.). Seventy-two patients (20 males and 52 females) aged 14–51 years had taken tetracycline 250 mg twice daily for 3–48 months (mean $9{\cdot}2 \pm 1{\cdot}2$ s.e.m.). Fifty-three patients on clindamycin had taken tetracycline previously. All patients were assessed for diarrhoea and pseudomembranous colitis. Full blood count and liver function tests (serum bilirubin and glutamic pyruvate transaminase) were estimated. No patient had a proctoscopy or sigmoidoscopy since there was no clinical indication for such an investigation.

RESULTS

The patients were matched for age and sex. No patient taking tetracycline had diarrhoea whereas nine patients on clindamycin developed diarrhoea ($P < 0{\cdot}0025$). In these nine patients bowel action increased to 2 to 9 times (mean 4) the normal daily rate and their stools were softer. This mild diarrhoea stopped within 2–4 weeks after withdrawing clindamycin. No patient either felt ill or developed pseudomembranous colitis. One patient on clindamycin had neutropenia but this followed an upper respiratory tract infection and the white cell count returned to normal value after a few weeks. No abnormality of liver function was noted although one patient complained that while on clindamycin he could take less alcohol.

DISCUSSION

Diarrhoea has been reported in 5–50% of patients on clindamycin or lincomycin (Duncan & Jeans, 1965; Breese *et al.*, 1966; Price *et al.*, 1968; Geddes *et al.*, 1970; Tedesco *et al.*, 1974). Although 12% of our patients on clindamycin had diarrhoea, no side-effects were seen in patients on tetracycline. Fifty of our patients on clindamycin had previously had tetracycline and so it is possible that their longer exposure to antibiotic had altered the gut bacterial flora. However, of the twenty patients who took clindamycin from the start of the treatment, three (15%) also had diarrhoea suggesting that a previous exposure to tetracycline is not essential for the development of diarrhoea. The definition of diarrhoea is difficult but our patients only had a mild alteration of bowel action and none had pseudomembranous colitis.

The reported incidence of pseudomembranous colitis has varied from 1 in 118,000 to 10% of 200 patients, 5% being the mean (Benner & Tellman, 1970; Viteri, Howard & Dyck, 1974; Tedesco *et al.*, 1974; McCleod, 1975). Pseudomembranous colitis usually occurs in patients with serious underlying diseases such as subacute bacterial endocarditis (Scott *et al.*, 1973) and many patients have had other antibiotics such as ampicillin and tetracycline which may cause pseudomembranous colitis (Finland, Grigsby & Haight, 1954; Schapiro & Newman, 1973). The incidence of pseudomembranous colitis due to clindamycin is probably no greater than that due to tetracycline (Klotz, Palmer & Kirsner, 1953; Reiner, Schlesinger & Miller, 1952).

We only use clindamycin as follows:

1. Moderate to severe acne which has failed to respond adequately to tetracycline, erythromycin and cotrimoxazole and topical applications.
2. Moderate to severe acne harbouring *C. acnes* which are sensitive to clindamycin and resistant to tetracycline, erythromycin and cotrimoxazole. This is uncommon.

Having prescribed clindamycin we warn the patients about diarrhoea and suggest that if there is an increase in their bowel action of more than one per day they should stop the treatment. This is an arbitrary but practical criterion.

Thus, although clindamycin may cause intestinal abnormalities it is an effective alternative to tetracycline (Cunliffe and Cotterill, 1973) and cotrimoxazole (Cotterill *et al.*, 1971). As long as we remember the unwanted effects and keep the patients under review, clindamycin should not be withheld from those with severe acne which fails to respond to tetracycline, erythromycin and cotrimoxazole.

REFERENCES

BENNER, E.J. & TELLMAN, W.H. (1970) Pseudomembranous colitis as a sequel to oral lincomycin therapy. *American Journal of Gastroenterology*, **54**, 55.

BREESE, B.B., DISNEY, F.A., TALPEY, W.B. & ROCHESTER, N.Y. (1966) Beta-haemolytic streptococcal illness—comparison of lincomycin, ampicillin and potassium penicillin G in treatment. *American Journal of Diseases of Childhood*, **112**, 21.

COTTERILL, J.A., CUNLIFFE, W.J., FORSTER, R.A., WILLIAMSON, D.M. & BULUSU, L. (1971) A comparison of trimethoprim sulphamethoxazole with oxytetracycline in acne vulgaris. *British Journal of Dermatology*, **84**, 366.

CUNLIFFE, W.J., COTTERILL, J.A. & WILLIAMSON, B. (1972) The effect of clindamycin in acne—A clinical and laboratory investigation. *British Journal of Dermatology*, **87**, 37.

CUNLIFFE, W.J. & COTTERILL, J.A. (1973) Clindamycin as an alternative to tetracycline in severe acne vulgaris. *The Practitioner*, **210**, 698.

DUNCAN, I.B.R. & JEANS, B. (1965) Lincomycin in hospital practice. *Canadian Medical Association Journal*, **93**, 685.

FINLAND, M., GRIGSBY, M.E. & HAIGHT, T.H. (1954) Efficacy and toxicity of oxytetracycline (Terramycin) and chlortetracycline (aureomycin). *Archives of Internal Medicine*, **93**, 23.

GEDDES, A.M., BRIDGEWATER, F.A.J., WILLIAMS, D.N. OON, J. & GRIMSHAW, G.J. (1970) Clinical and bacteriological studies with clindamycin. *British Medical Journal*, **ii**, 703.

KLOTZ, A.P., PALMER, W.L. & KIRSNER, J.B. (1953) Aureomycin proctitis and colitis: a report of five cases. *Gastroenterology*, **25**, 44.

MACLEOD, N. (1975) Personal communication.

PRICE, D.J.E., O'GRADY, F.W., SHOOTER, R.A. & WEAVER, P.C. (1968) Trial of phenoxymethyl penicillin, pheneticillin and lincomycin in treatment of staphylococcal sepsis in a casualty department. *British Medical Journal*, **iii**, 407.

REINER, L., SCHLESINGER, M.J. & MILLER G.M. (1952) Pseudomembranous colitis following aureomycin and chloramphenicol. *Archives of Pathology*, **54**, 39.

SCHAPIRO, R.L. & NEWMAN, A. (1973) Acute enterocolitis. *Radiology*, **108**, 263.

SCOTT, A.J., NICHOLSON, G.I. & KERR, A.R. (1973) Lincomycin as a cause of pseudomembranous colitis. *Lancet*, **ii**, 1232.

TEDESCO, F.J., BARTON, R.W. & ALPERS, D.H. (1974) Clindamycin associated colitis. *Annals of Internal Medicine*, **81**, 429.

VITERI, A.L., HOWARD, P.H. & DYCK, W.P. (1974) The spectrum of lincomycin-clindamycin colitis. *Gastroenterology*, **66**, 1137.

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Case Reports

Hormonal Levels in Fox-Fordyce disease

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It is now 70 years since this condition was first described (Fox & Fordyce, 1902). Hormonal factors have been implicated in the aetiology (Shelley & Levy, 1956; Montes *et al.*, 1961) and when a case of the disorder was seen recently the opportunity was taken to investigate the endocrine background by means of recent techniques (Mayes & Nugent, 1968; Norymberski, 1952; Horning *et al.*, 1963; Kirschner & Lipsett, 1963; Cox, 1959).

CASE REPORT

Miss L.P., aged 15 years, presented with a 6-month history of an itchy rash affecting both axillae and also the circumareolar area of both nipples. The clinical appearance suggested Fox–Fordyce disease. Biopsy showed: 'a chronic inflammatory infiltrate surrounding the dilated apocrine glands, as well as hair follicles and blood vessels. Spongiosis was noted in the epidermis and within the lining of the hair follicles'.

Urine vol 24 h 670 ml

	mg/24 h	Range	Mean value for age 15	
17-Oxosteroids	5·3	1·5–11	4·5	
17-Oxogenic steroids	5·0	3–13	6·5	
Androsterone	1·9	0·9–2·6		
Aetiocholanolone	1·1	0·6–2·2		
Dehydroepiandrosterone	0·1	0·2–1·1		
11-Ketoaetiocholanolone	0·4	0·3–0·9		
11-Hydroxyandrosterone	0 5	0·1–0·8		
11-Hydroxyaetiocholanolone	0·2	0·1–1·2		
Acetaldehydogenic steroids			Child	Women
Pregnanetriol	0·9		0·0 5	0·2–1·5
Pregnanetriolone	0·2		0·05	0·005
5-Pregnenetriol	0·4		0·05	0·05–0·3
Pregnanetetrol	None detected		Not established	

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* Ethinyloestradiol 0·1 mg: Ethinyloestradiol 0·1 mg+Megestrol acetate (marketed as Serial-C., B.D.H. Pty. Ltd.).

The patient was given a single dose of 90 rads of superficial X-ray to each axilla (HVL 1·5 mm aluminium). She was also given a 3-months course of oral sequential contraceptive tablets.* Both these measures were without effect.

It was pointed out to the patient that the only other form of treatment likely to be effective would be radical excision of the axillae and replacement by split skin. The patient and her parents, after considering this, came to the conclusion that the symptoms were not bad enough to warrant such an extensive procedure. She has been followed for over 2 years and seems to be adapting to the itchiness quite well, except in periods of emotional tension.

Laboratory studies

Plasma testosterone, 24 ng/100 ml (normal 20–76).

CONCLUSIONS

These results do not disclose any evidence of significant hormonal abnormality. The slight elevation in pregnanetriolone and delta 5-Pregnenetriol levels may be incidental or could be as an indication for similar investigations on other patients with this disorder.

REFERENCES

Cox, R.I. (1959) The separation and quantitative estimation of pregnane 3α, 17α, 20α-triol, pregnane 3α, 17α, 20α-triol-11-one, and other urinary acetaldehydogenic steroids. *Journal of Biological Chemistry*, **234**, 1693.

Fox, G. & Fordyce, J. (1902) Two cases of a rare papular disease affecting the axillary region. *Journal of Cutaneous Diseases*, **20**, 1.

Horning, E.C., Luukkainem, T., Haahti, E.O.A., Creech, B.G. & Van den Heuvel, A.J.A. (1963) Studies of human steroidal hormones by gas chromatographic techniques. *Recent Progresss in Hormone Research*, **19**, 57.

Kirschner, M.A. & Lipsett, M.B. (1963) Gas–liquid chromatography in the quantitative analysis of urinary 11-Deoxy-17-ketosteroids. *Journal of Clinical Endocrinology*, **23**, 255.

Mayes, D. & Nugent, C.A. (1968) Determination of plasma testosterone by the use of competitive protein binding. *Journal of Clinical Endocrinology*, **28**, 1169.

Montes, L.F., Caplan, R.M., Riley, G.M. & Curtis, A.C. (1961) Fox–Fordyce disease. An endocrinological study. *Archives of Dermatology*, **84**, 452.

Norymberski, J.K. (1952) Determination of urinary corticosteroids. *Nature*, **170**, 1074.

Shelley, Walter B. & Levy, Edwin, J. (1956) Apocrine sweat retention in man. *Archives of Dermatology*, **73**, 38.

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Lichen scrofulosorum

A REPORT OF FOUR CASES

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SUMMARY

Four cases of lichen scrofulosorum associated with lymph node tuberculosis are reported, and the clinical and histological appearances of the disorder are described.

Lichen scrofulosorum was first recognized by Hebra and his description is a model of precision and clarity. In a footnote to the English translation (1868) the editors commented that '. . . English writers seem generally unable to identify the lichen scrofulosorum with any disease observed by themselves . . .'. To later generations of dermatologists it became a readily recognized but uncommon entity which was associated with tuberculosis of lymph nodes or bone, although the infection was not always obvious at the time of development of the rash (Miescher, 1955). The eruption characteristically occurs in children and young adults. All Hebra's cases were males under 25 years old. Volk (1931) suggested that women may be more commonly affected in the older age group. Other forms of cutaneous tuberculosis might at times be present in the same patient. Phlyctenular conjunctivitis was present in 50% of patients in one series (Rauschkolb, 1934). Lichen-scrofulosorum-like lesions have also been seen following B.C.G. vaccination (Dostrovsky & Sagher, 1963).

There have been few case reports under this title in the last two decades. The situation has been somewhat confused by the description of 'lichenoid tuberculid', an apparently heterogeneous disorder (Ockuly & Montgomery, 1950). Some of the original cases described by Ockuly & Montgomery may have been cutaneous sarcoidosis. A rather extreme position is taken by Lever (1967) who concluded from the clinical and histological data that lichen scrofulosorum is identical with the lichenoid type of sarcoidosis. We are reporting the following four cases to rectify this erroneous suggestion and to show how well the condition responds to antituberculous treatment.

CASE REPORTS

Case 1

A 27-year-old nurse, born in Vietnam but living for 5 years in Britain, was seen with a symptomless rash on the trunk which had been gradually spreading for 1 year. Her past health had been good apart from an episode of headache and vomiting 4 months previously, when her submandibular and cervical lymph nodes were swollen. Some swelling of the cervical nodes had probably been present for 6 years. The patient's maternal grandmother had been treated for pulmonary tuberculosis 4 years previously. Examination showed an eruption consisting of follicular skin-coloured papules, 1–2 mm in diameter, on the lower chest and abdomen with a tendency to grouping. The left submandibular and upper cervical lymph nodes were enlarged.

Investigations. Chest X-ray showed an old calcified tuberculous focus in the right mid-zone, but no evidence of an active lesion. ESR was 40 mm/h (Westergren). The Mantoux reaction (1:10,000) was strongly positive, producing induration and swelling of the whole forearm within 48 h. Twelve days after the Mantoux test a crop of lichenoid papules identical to the original eruption developed over the test-site area. Biopsy of one of the enlarged cervical nodes showed tuberculous histology; fluorescent microscopy and culture were positive for *Myobacterium tuberculosis*. Skin biopsy showed some parakeratosis in the region of the hair follicles, with follicular plugging. There was an upper dermal granulomatous infiltrate of epithelioid cells closely surrounding the follicular ducts. Some Langhans giant cells were present in the infiltrate and there were lymphocytes at the periphery. No acid-fast bacilli were seen in the sections examined and none could be cultured from the skin.

The patient was treated with isoniazid 300 mg/day, ethambutol 1000 mg/day and pyridoxine 10 mg/day. After 7 weeks the dose of ethambutol was reduced to 600 mg/day. After 4 months there was marked reduction in the size of the cervical glands and the skin eruption had cleared completely.

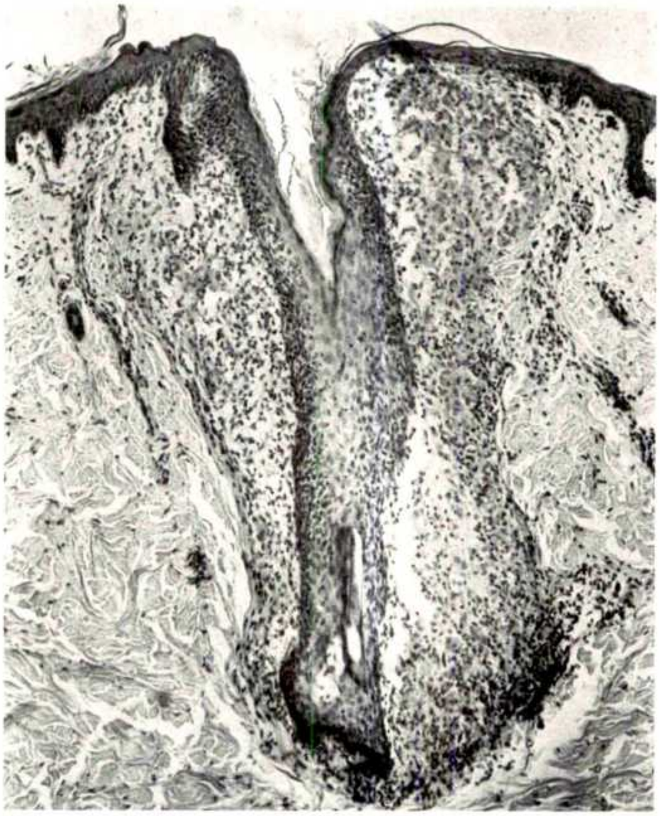

FIGURE 1. Low power view of lesion from case 2, showing a granulomatous infiltrate of epithelioid cells and lymphocytes surrounding a hair follicle (H & E, ×83).

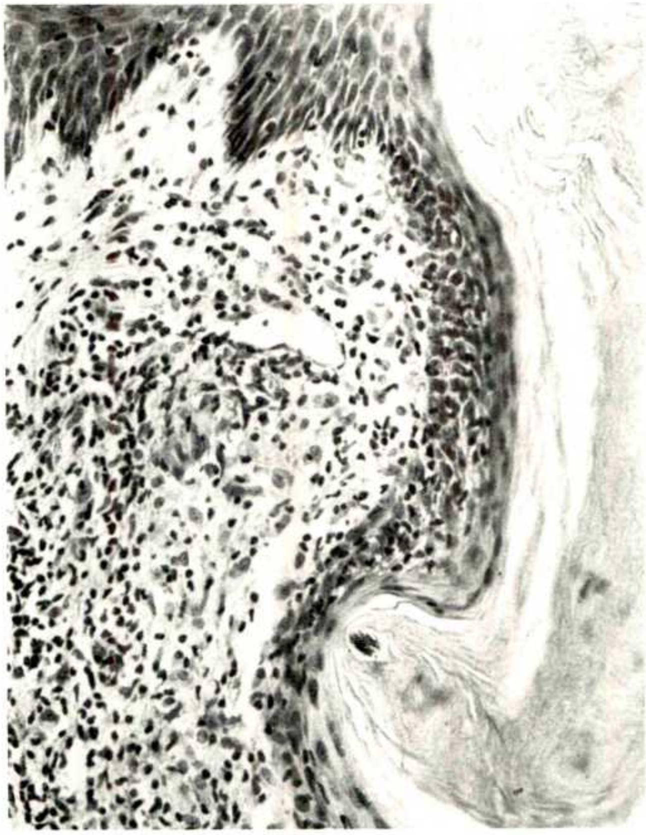

FIGURE 2. Case 2, high power view, showing a perifollicular non-caseating tuberculoid granuloma (H & E, × 208).

Case 2

An 18-year-old Tanzanian male student had had a rash affecting the trunk and upper arms for 2 months. For 6 weeks there had been a rapidly progressive enlargement of the lymph nodes on both sides of his neck, and he had had a low grade fever. There was no relevant past medical history and no family history of tuberculosis. A buff-coloured lichenoid rash was patchily distributed over the trunk and upper limbs. There was some follicular hyperkeratosis and the individual lesions were up to 3 mm in diameter. Bilateral cervical lymphadenopathy was present. The nodes were soft, fluctuant and fixed to deeper tissues.

Investigations. Chest X-ray showed enlargement of the left hilar and right paratracheal nodes. ESR was 46 mm/h. Mantoux reaction (1 : 10,000) was strongly positive with 12 mm of induration and blister formation. Biopsy of cervical and nasopharyngeal lymph nodes showed typical tuberculous histology. Tubercle bacilli were identified both on direct smear and culture of the pus from a cervical node. Histology of the skin lesions (Figs 1 & 2) showed non-caseating tuberculoid granulomata both in the upper dermis and surrounding hair follicles. No acid-fast bacilli were seen.

The patient was treated with streptomycin 0·75 g/day, sodium aminosalicylate 12 g/day and isoniazid 300 mg/day. After 7 weeks the skin eruption was much less obvious, chest X-ray revealed reduction in size of the hilar lymph nodes and the ESR had fallen to 10 mm/h. After 11 weeks the skin was clear and there was no cervical lymphadenopathy although on chest X-ray there was still some enlargement of the left hilar nodes.

Case 3

A 66-year-old English woman developed a rash 6 years after receiving treatment for lymph node tuberculosis. When she was first seen she had had symptoms of dysphagia, a persistent cough and night sweats. Enlarged supraclavicular lymph nodes were found on examination. Biopsy of the nodes showed the presence of tuberculoid granulomata, and *Mycobacterium tuberculosis* was cultured from the biopsy material. *M. tuberculosis* was also cultured from the sputum on one occasion but the chest X-ray then showed only some paralysis of the left diaphragm with no evidence of active pulmonary tuberculosis. The patient was treated with calcium benzamidosalicylate and isoniazid for 1 year, and streptomycin which was stopped after 5 weeks because of tinnitus. The lymphadenopathy disappeared, and the patient was free of symptoms for 6 years. She then developed discomfort in the right eye and was treated for an anterior uveitis. A few months later she developed a symptomless rash over the back, chest and abdomen. Though there was some spontaneous regression of the rash during the following 12 months, after about 18 months the eruption recurred in a more severe form.

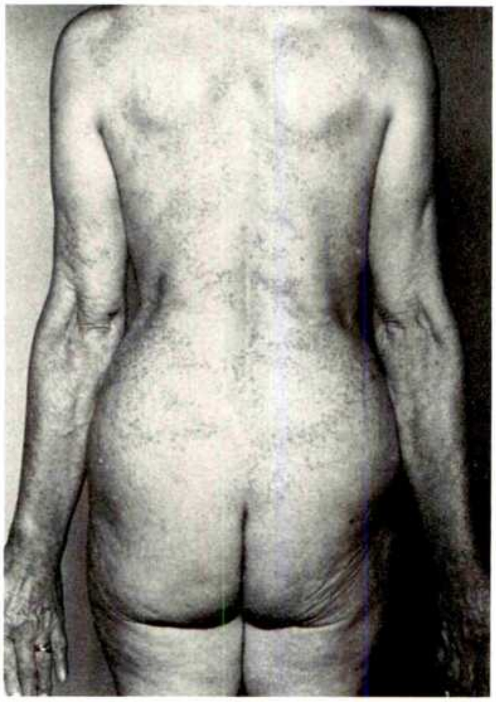

FIGURE 3. Case 3, distribution of the lesions over the back.

Examination showed a pinkish-brown grouped micropapular rash over the trunk and back (Fig. 3), individual lesions being 2–4 mm in diameter. There was no evidence of lymphadenopathy and examination of the eyes showed the appearances of a quiescent right uveitis with posterior synechiae formation.

Investigations. Chest X-ray showed slight residual weakness of the left diaphragm. ESR was 26 mm/h. Mantoux reaction (1:10,000) was positive, producing 12 mm of induration. A Kveim test was negative. Two skin biopsies were taken during the course of the illness, both showing essentially similar features. The epidermis was slightly thinned and there was some oedema of the papillary dermis. Non-caseating epithelioid cell granulomata were present in the upper dermis adjacent to hair follicles in some areas. A few giant cells were seen and there was a patchy peripheral lymphocytic infiltrate.

The patient was treated with rifampicin 450 mg/day and isoniazid 300 mg/day. After 3 months of treatment the skin eruption had cleared completely.

Case 4

A 53-year-old Englishman was seen in the Skin Department of the Royal Free Hospital in 1969 with a 6-week history of a widespread eruption on the trunk and limbs. Nine years previously he had been discovered to be suffering from tuberculosis of a cervical node on the left side of the neck and was treated with streptomycin and sodium aminosalicylate/isoniazid (Pasinah, Wander) for 2 years. At that time, the diagnosis of tuberculosis was confirmed by the finding of acid-fast bacilli in the pus, and guinea-pig inoculation was also positive. In 1964 the lump in the neck reappeared and a further 1 year's treatment with sodium aminosalicylate was given. He was well until 1968 when the lymphadenopathy recurred and he was treated with streptomycin for 3 months and Pasinah for 7 months. In January 1969, while on antituberculous treatment, the lesion in his neck became swollen and tender and surgical drainage of a collar-stud abscess was carried out. In May 1969 a diffuse lichenoid eruption appeared on his trunk and limbs. He was off all antituberculous therapy at that time. The patient's mother had a history of diabetes and tuberculosis and a brother aged 37 had died of tuberculosis. On examination, he was found to have many discrete, in some parts almost confluent, lichenoid papules (Fig. 4) resembling those of lichen planus, but less shiny and less violaceous in colour, over the chest, abdomen, back, buttocks and limbs. There was a string of swellings in the left posterior triangle of the neck and he had a left hydrocele.

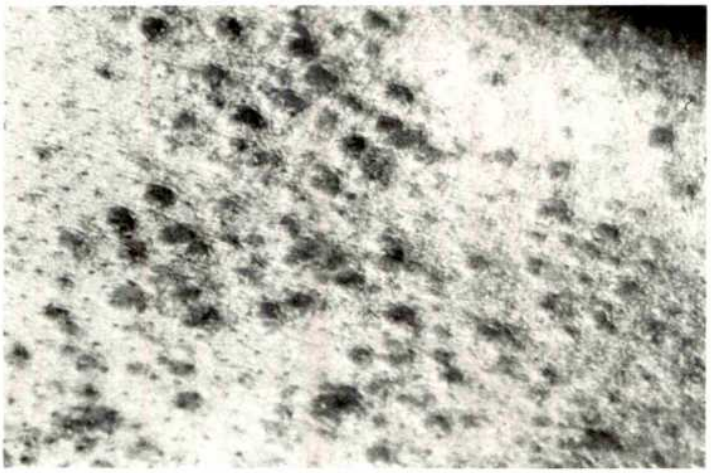

FIGURE 4. Case 4, detail of the lesions on the back.

Investigations. Chest X-ray showed pleural thickening and bilateral apical scarring. There was also a density in the left upper zone which was thought to be due to old tuberculosis. A Mantoux test (1:100,000) was positive. Histology of the skin from the left scapular region showed several non-caseating granulomata in the dermis. No acid-fast bacilli were seen in the section, and none were cultured from the cervical lymph node.

A diagnosis of lichen scrofulosorum was made and in view of continued discharge from his neck, a course of sodium aminosalicylate and isoniazid (Inapasade, Smith & Nephew Pharmaceuticals) was started. Within 2 weeks the rash was much less obvious, and 1 month later it had disappeared altogether.

DISCUSSION

The four cases described correspond well to Hebra's original description of lichen scrofulosorum. All the patients had lymph node tuberculosis, the skin lesions are comparable in clinical appearance and distribution and in all cases the eruptions appear to have improved or resolved completely within months of commencing antituberculous therapy.

The cutaneous lesions of lichen scrofulosorum are sufficiently characteristic for a diagnosis to be suspected on clinical grounds alone. Typically the eruption consists of symptomless papules, usually follicular in distribution and 0·5–3 mm in diameter. Although usually skin-coloured, they may vary from yellow to reddish-brown. The papules occur in groups sometimes with a patchy, annular arrangement and are most characteristically seen on the abdomen, chest and back. There may be some scaling over the lesions and micropustule formation has been described. Skin lesions somewhat resembling those of lichen scrofulosorum may occasionally be seen in other conditions. The papules of lichen nitidus tend to be shiny and commonly occur on the limbs and genital area as well as the trunk. Keratosis spinulosa is a condition which may be indistinguishable from lichen scrofulosorum clinically although the cutaneous lesions tend to be less inflammatory with more obvious follicular hyperkeratosis and rarely show the histological appearances of lichen scrofulosorum. Limb and face involvement is common in the papular and lichenoid types of cutaneous sarcoidosis and the Mantoux reaction should help to distinguish the two conditions. In lichen scrofulosorum the Mantoux reaction is always positive and usually strongly so. Fungal infections of the skin may produce patchy annular eruptions but small follicular papules are seldom seen. Secondary syphilis and lichenoid drug eruptions may occasionally produce a picture superficially similar to that of lichen scrofulosorum.

The typical histological appearances of lichen scrofulosorum (Montgomery, 1937) consist of superficial dermal granulomata, usually surrounding hair follicles and sweat ducts, but occasionally independent of either. The infiltrate is composed of epithelioid cells with some Langhans giant cell formation and a narrow margin of lymphocytes at the periphery. The adjacent capillaries may show a lymphocytic perivascular infiltrate and there may be non-specific secondary changes in the epidermis above the lesions (Johnson, 1972). There is no caseation necrosis and changes are not seen in the subcutaneous tissue. Normally tubercle bacilli are not seen in the pathological specimens, nor can they be cultured from biopsy material. Some of the earlier writers, however, have claimed to identify bacilli in the skin lesions (Volk, 1931). The distribution of the granulomata around the appendages and the fact that the infiltrate often occupies several dermal papillae are helpful in differentiating lichen scrofulosorum from lichen nitidus. The cutaneous lesions of secondary syphilis are essentially perivascular and plasma cells are frequently seen in the infiltrate. In cutaneous sarcoidosis the granulomata tend to be more discrete with less surrounding lymphocytic infiltrate, but distinction of sarcoidosis from lichen scrofulosorum may be impossible on histological grounds alone.

The course of the untreated disease tends to be slow, the lesions persisting for months or years. Spontaneous involution without scarring usually occurs eventually. In all our cases there has been an excellent response to antituberculous treatment, although not all writers have had the same experience (Schuhmachers, 1967).

There is little doubt that tuberculous infection is an aetiological factor in the development of the cutaneous lesions of lichen scrofulosorum. The exact nature of the relationship between systemic tuberculosis and the skin eruption is not completely clear. At one time the cutaneous lesions were considered to be reactions to 'toxins' of tubercle bacilli. It is now thought more likely that they result from haematogenous dissemination of bacilli in an individual strongly sensitive to *Mycobacterium tuberculosis*, though the primary focus of dissemination may not always be apparent.

Lichen scrofulosorum appears to be a definite clinical and pathological entity, distinct from other forms of cutaneous tuberculosis and unrelated to sarcoidosis. Its prevalence in any community probably depends upon the prevalence of tuberculosis of the lymph nodes and it is felt that the condition is perhaps not so uncommon as the lack of recently documented cases would suggest.

ACKNOWLEDGMENTS

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Case 3 was presented by Dr J.P.Ellis at a meeting of the Dermatological Section of the Royal Society of Medicine on 21 February 1974, and was subsequently listed in the Proceedings of the Royal Society of Medicine.

REFERENCES

Dostrovsky, A. & Sagher, F. (1963) Dermatological complications of B.C.G. vaccination. *British Journal of Dermatology*, **75**, 181.

Hebra, F. (1868) Lichen scrofulosorum. In: *Diseases of the Skin* (Translated and ed. by C.H.Fagge and P.H.Pye-Smith), Vol. 2, p. 52. The New Sydenham Society, London.

Johnson, W.C. (1972) Lichen scrofulosorum. In: *Dermal Pathology* (Ed. by J.H.Graham, W.C.Johnson and E.B.Helwig), p. 394. Harper & Row, Hagerstown, Maryland.

Lever, W.F. (1967) In: *Histopathology of the Skin*, 4th. edn, p. 301. Pitman Medical, London.

Miescher, G. (1955) Über katamnestische Untersuchungen bei Fällen mit Tuberkulid. *Dermatologica*, **110**, 23.

Montgomery, H. (1937) Histopathology of various types of cutaneous tuberculosis. *Archives of Dermatology and Syphilology*, **35**, 698.

Ockuly, O.E. & Montgomery, H. (1950) Lichenoid tuberculid. *Journal of Investigative Dermatology*, **14**, 415.

Rauschkolb, J.E. (1934) Tuberculosis of the skin: a clinical report. *Archives of Dermatology and Syphilology*, **29**, 398.

Schuhmachers, R. (1967) 2 Fälle eines lichenoides Tuberkulids (= Lichen scrofulosorum). *Hautarzt*, **18**, 81.

Volk, R. (1931) Lichen scrofulosorum. In: *Handbuch der Haut-und Geschlechtskrankheiten* (Ed. by J. Jadassohn) Vol. 10, p. 331. Springer, Berlin.

British Journal of Dermatology (1976) **94**, 327.

History of Dermatology

Some aspects of the genesis and development of German dermatology

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The use of the expression 'Ritterburg' (a Knight's Castle) for a skin clinic is familiar to older German dermatologists and calls to mind the way venereal disease was ostracized by a former society which considered it to be entirely the responsibility of the affected individual. This attitude was reflected not least in the tardy provision of insufficiently equipped buildings and premises to be used for treatment. In Germany, however, unlike most other countries at that time, venereal disease and dermatology were, at least in the universities, almost without exception combined as one specialty.

Nevertheless, it is not so long since these two subjects actually rose to university level, for it is only since about 1912 that most German universities have had at their disposal a specialist with adequate premises. However, in Germany, most of the very earliest beginnings can be dated back to the late Middle Ages. The genesis of venereology, which was a field related more to surgery, can be placed in the sixteenth century, whereas for dermatology, which developed from general medicine, the sixteenth and eighteenth centuries are more appropriate. Moreover, it was not uncommon for venereology and laryngology on the one hand, and for dermatology and paediatrics on the other, to be linked together.

Then, as is still the case in a sub-specialty, the young graduate would, in the field of dermatology and venereology, busily undertake to lecture on such subjects as would not feature in the dermatological material of the main lectures given at some time or other by the directors of the internal clinics and policlinics. It is more remarkable that the introduction of dermatovenereology as a subject in the medical state examination of 1918 was demanded in Prussia by the army (and, among others, by the then Governor General of Belgium, von Bissing, in his capacity as a member of the 'Herrenhaus').

Hildegard von Bingen, who was born in 1089 near Mainz and died in 1179 as the Abbess of the convent she founded in Rupertsberg, had some knowledge of the scabies mite; this was 800 years ago and long antedated the days of Bonomo, Renucci or von Hebra. Saint Hildegard, on two different occasions, mentioned 'suren', the word in use at that time for sarcoptes scabiei.

As an eminent figure within the German cultural circle of the Middle Ages, *Samuel Hafenreffer* should be mentioned here. He was the author of the *first German textbook of dermatology*, although it was written in Latin. He was born in Herrenberg in 1587, practised for a time in Tübingen and finally in 1660, he died in harness as the town physician for Ulm. His book, entitled '*παντοδοχεῖον αἰλοδερμον*' which in English means something like '*The Inn of the Gay Fleece*', first appeared in 1630 and was followed by a second edition ('Nosodochion') in 1660.

As a bizarre example of the venereological teaching at that time, the following may be quoted:

'Cure of gonorrhoea through coitus with a virgin'—'Hoc quoque scio Antiqua Gonorrhoea plures fuisse liberatos, qui cum virgine rem habuerunt. . . .'

However, the *first dermatological textbook* to be written *in German* was by *Conrad Fuchs* who had been a pupil of *Johann Lucas Schönlein*, and was Professor at Göttingen. It was entitled *'Die krankhaften Veränderungen der Haut und ihrer Anhänge'*, and was published first in 1840 and 1841. This work was still based on a botanical classification of dermatological manifestations. *Schönlein*, who was born in 1793 in Bamberg and died there in 1869, was one of the most prominent German clinicians of modern times; he was the first resolutely to deliver his course of lectures in German instead of Latin. He is regarded as the founder (with, amongst others, Robert Remak) of a school of natural history, and apart from his thesis, the published scientific works he left behind consist merely of about three pages. Of particular importance to the dermatologist was his original account of the organism in favus, whereupon the disease lost its former significance as being an 'hereditary' scurf. This original description went under the title *'Zur Pathogenie der Impetigines'*, and took up just 23 lines in the *'Archiv für Anatomie'* by Johann Müller (p. 82) published in 1839. It was in this same year that another pathogenic microorganism was discovered, that of thrush (Candida) found by Bernhard von Langenbeck (1810–87) in the intestine of a child that had died of typhoid. However, the expression Schönlein's purpura, familiar to every dermatologist, was recorded only in a college obituary 'by some of his students' in 1866. Worthy of especial mention from the dermatologists' point of view is *Johann Ernst Wichmann* (1740–1802) of Hannover, who was one of the most famous German physicians of the second half of the eighteenth century. He not only confirmed the parasitic 'Ätiologie der Krätze' ('Aetiology of Scabies'), which was published in 1786, but also described particularly well the clinical appearance of *'crusta lactea'* and of *'Pemphigus chronicus'* in 'Beytrag zur Lehre des Pemphigus', published in Erfurt in 1791.

Berlin

Around the middle of the nineteenth century, the evolution of dermatovenereology took place very rapidly in Berlin, thanks to *Friedrich Wilhelm von Bärensprung*. He, like his predecessor (*Carl Gustav Theodor Simon* (1810–57) whose works included studies on condylomata and warts, and on demodex folliculorum), fell victim to progressive paralysis. In 1853 a department was provided for Bärensprung to use for the care of syphilitic patients, and in 1858 another was furnished for skin diseases at the request of Schönlein.

von Bärensprung, son of the then mayor of Berlin, devoted himself as did his contemporary Ludwig Traube to the study of precise thermometry. In this way, systematic temperature-taking in hospital, at least in Germany, was mainly a dermatological innovation. Bärensprung, being professor of dermatology and syphilology was able more precisely to describe as clinical entities many dermatoses such as erythema marginatum, erythrasma and plica polonica. Furthermore, he made the discovery that syphilis, gonorrhoea and chancroid were each the result of infection by a different organism. Especially noteworthy at that time was his stubborn refusal to approve the use of mercury to treat syphilis in his practice, a view which was shared later by the physician Kussmaul. He deserves especial merit for his research into herpes zoster (for instance in *Ann. Charité*, **9**, 40–128, 1861) in which he proved that the disease involves the spinal ganglia. Bärensprung's (presumed) sense of satisfaction was probably negated by his reproaching himself severely for the failure of his experiments with syphilis inoculations. This earned him, and later Rinecker of Würzburg and Neisser of Breslau as well, public hostility. Amongst his pupils was *Max Burchardt* (1831–97), who worked first as an army physician and subsequently as an ophthalmologist; he was notable for having described, in 1859, 'corynebacterium minutissimum' as the cause of *erythrasma*.

Bärensprung's successor, *Georg Lewin* (1820–96), also held courses in laryngoscopy and became well known for his practice of administering mercuric chloride intramuscularly. In 1884, he had to hand over his department for skin diseases to *Ernst Schweninger* (1850–1924), who later was to become von Bismarck's personal physician. Schweninger had qualified in morbid anatomy at Buhl's institute in Bavaria. He is well remembered by dermatologists, above all for being, with *S. Buzzi*, the first to describe a variety of *anetoderma* in 1891.

Edmund Lesser (1852–1918) was Lewin's successor and was able in 1902, on Schweninger's resignation, to reunite the whole department. In 1911, he became the first Berliner to hold the appointment of locum tenens 'ordinarius' in the specialty, being professor in ordinary.

The cause of syphilis was recognized in Lesser's day, the *Treponema pallidum* being discovered on 3 March 1905 by *Fritz Richard Schaudinn* (1871–1906) and his co-worker *Erich Hoffman* (1866–1959); the latter was an active military doctor drafted to the Charité clinic. Initially, the spirochaete had been demonstrated unstained by light microscopy. Later, in addition to Neisser and Bruck, *August Wassermann* (1866–1925) discovered in 1906 the serological test for diagnosing syphilis, which bears his name. However, the principal advance in the management of syphilis was made in 1909 when *Paul Ehrlich* (1854–1915) introduced his 'Ehrlich-Hata 606', which, since 1910, has been commercially available under the name of '*Salvarsan*'.

Lesser knew how to gather around himself a group of important pupils; one of them, Georg Arndt (1874–1929), became his successor.

Arndt held the lectureship in dermatology at Strasbourg from 1916, in succession to A.Wolff. (Because the period of German lectureship in Strasbourg was a temporary one, Burgun's and Roederer's papers should also be mentioned here). Anyone who ever came into personal contact with Arndt would praise him for the rest of his life as one of the greatest morphologists, even though his published work was rather meagre. Amongst other things he coined the term 'Brocq's disease'. Among his students may be counted H.Löhe and H.A.Gottron, but in particular Frau *Marie Kaufmann-Wolf* (1877–1922) who will always be remembered not only for her divers and painstaking clinical work (lymphocytoma and secondary carcinoma of the skin) but also for her discoveries in mycology. Arndt's contemporary in Berlin, *Oskar Lassar* (1849–1907), as well as being renowned for his Zinc Paste, was the founder and, from 1843–1907, the editor of the '*Dermatologische Zeitschrift*', which was subsequently edited by Erich Hoffman from 1908 to 1938, and now appears as 'Dermatologica'.

The management of the clinic was taken over, after Arndt, by Franz Blumenthal, who in turn was succeeded, in 1932, by *Walter Friboes* (1880–1945). The latter, during his time at Rostock, became in 1927 the first dermatologist to be appointed Rector of a university. The successive management of the professorial chair at the Charité after 1945 lay first in the hands of *Heinrich Löhe* (1877–1961) who was interested particularly in the venereological side; it then came under *K.Linser* (born 1895), and subsequently *Wolfgang Gertler* (born 1904) who has recently had published his impressive and morphologically extremely sound three-volume text 'Systematische Dermatologie' (Systematic Dermatology). He has now been replaced by *Niels Sönnichsen.*

Erich Langer (1891–1957) started off as an industrious leading dermatologist at the 'Free University of Berlin'; he had first become famous when he and his teacher *Abraham Buschke* (1868–1943) wrote, in 1926, 'Lehrbuch der Gonorrhoe'; Buschke had been the author of 'Scleroedema adultorum' in 1902. In 1946, Langer founded the '*Zeitschrift für Haut- und Geschlechtskrankheiten*', which has since 1957 been run by H.Grimmer, the chief physician at Wiesbaden and who now also edits '*Mykologie*'.

Langer's successor at the clinic has been, since 1958, *H.W.Spier* (born 1912), who works primarily in the field of fundamental biochemistry in relation to dermatology; *Gunter Stüttgen* (born 1919), the director of the department of dermatology at the Rudolf Virchow Hospital of the 'Free University', has similar interests.

Breslau

Despite the older traditions of Berlin, Munich and Würzburg, the skin clinic at Breslau (now Wroclaw), at that time, already had the reputation of being the most prominent of its kind. This clinic was founded by *Heinrich Köbner* (1838–1904); he was forced, however, on account of ill health to hand it over on its opening day in 1877 to his successor. Köbner's name remains well known through his original description of epidermolysis bullosa hereditaria and his discovery of the isomorphic response of the skin to injury, now named the Köbner phenomenon. His successor, *Oskar Simon* (1845–1882), made a name for himself by his accounts of the histology of molluscum contagiosum, of the localization of skin diseases, and of taches bleues. Simon's successor, *Albert Neisser* (1855–1916), moved into the first German skin clinic to be designed and constructed as a complete separate building; this was opened in the Maxstrasse in 1892. Neisser became, in 1907, personal professor in ordinary; in 1912, he was made scheduled ordinarius. In 1888, he and E.R.Pick (1834–1910), who was the first Professor in ordinary in his subject in Austria, founded the '*Deutsche Dermatologische Gesellschaft*' (German Dermatological Society); moreover, in 1912, he founded the '*Deutsche Gesellschaft zur Bekämpfung der Geschlechtskrankeiten*' (German Society for the Fight against Venereal Diseases). He co-edited with F.J.Pick, from Volume 76 (1905) onwards, the '*Archiv für Dermatologie und Syphilis*'. This journal had been founded in 1869 by Auspitz and Pick, and it is now known as the '*Archiv für Dermatologische Forschung*' (Archives for Dermatological Research). Scientifically, Neisser's name is connected not only with the discovery of the cause of gonorrhoea—*Neisseria gonorrhoeae*—which his class-mate Ehrlich had called the gonococcus, but also with the use of aniline dye to stain and thus to demonstrate the lepra becillus which A.Hansen had originally discovered. Neisser also introduced the therapeutic use of Tumenol (ammonium sulfobituminate) and Protargol. Long before the concept of allergy had been formulated, he, and his assistant *Plato*, suggested studies of *trichophytin* (1902). Other members of the Neisser dynasty worthy of mention include: C.Bruck (*WR*), E.Galewski (Consultant at Dresden; Cignolin (dithranol)), F.Juliusberg and V.Klingmüller (Kiel), as well as E.Kuznitzky, who, in 1915 with the general physician A.Bittorf, described lung involvement in sarcoidosis.

Joseph Jadassohn (1863–1936) was called to Breslau to succeed Neisser. He had been working at Berne from 1896 to 1917, and while there had, in 1904, become the first professor in ordinary of Switzerland. He is well known not only for his work on odol-eczema, and on anetoderma (1890) which he so named, but also for his comprehensive descriptions of pityriasis lichenoides chronica, cutis verticis gyrata, granulosis rubra nasi (in 1901), pachyonychia congenita, 'trichophytide', as well as blue naevus. Apart from contributions to various handbooks (Mraček, Lesser, Epstein-Schwalbe), his own 23-volume handbook of skin and venereal diseases ('*Handbuch der Haut- und Geschlechtskrankheiten*') was published during the period 1927–34. Furthermore, in 1921, he and W.Pick started the '*Zentralblatt für Haut- und Geschlechtskrankheiten*'. J.Jadassohn was followed by *Max Jessner* (whose most important pupil was *Stephan Epstein*), and he in turn was followed by H.A.Gottron who held the post until 1945.

Of the famous pupils of Jadassohn worthy of mention eclectically are Lewandowsky (Basel), B.Bloch (Zürich), Biberstein, Martenstein, Freudenthal, and Hermann Pinkus. Wilhelm Frei, who was physician director at the Municipal Hospital of Berlin-Spandau from 1929, gave his name to the test he described for lymphogranuloma inguinale.

Würzburg

Even before the days of Schönlein, the early beginnings of dermatovenereological training were underway at Würzburg, but it was only under *Franz von Rinecker* at the Julius Hospital (1811–83) that these were moulded solidly and brilliantly. After Rinecker's death (and he was, moreover, to undertake a teaching assignment in psychiatry), dermatology returned to the care of the general physicians (Gerhardt). The first exclusively dermatological specialist was *Karl Zieler* (1874–1945), who had received his training from Doutrelepont and Neisser; he became active in Würzburg from about 1909, becoming ordinarius in 1921.

His special interests were the management of skin tuberculosis and the treatment of syphilis. In addition to his extensive casuistry, he left many works which are highly treasured even now. These include his monographs, especially his 'Atlas der Hautkrankheiten' produced in association with E.Jacobi, his 'Handbuch der Salvarsantherapie', written with Kolle, and the five-volume '*Handbuch der Haut-. und Geschlechtskrankheiten*' co-edited with Leopold Arzt.

Also important in the history of dermatology at Würzburg at that time was the elucidation by the surgeon *Friedrich Fehleisen* (1854–1929) of the aetiology of *erysipelas*, and the description by the dermatologist *Max Bockhart* (1887) of *staphylococcal follicular impetigo*; Bockhart later settled in Wiesbaden. *Karl Hoede* (1897–1974) had been working at the Würzburg Clinic from 1926 and became Ziegler's successor from 1940–45. His accounts included tuberculosis of the skin, the genodermatoses, and especially the role of heredity in psoriasis. *Hans Schuermann* ran the clinic between 1948 and 1958 and was followed, in 1959, by *Arthur Leinbrock* who held the position until 1964. Since 1965, *Helmut Röckl* (born 1920) has been in charge of the clinic.

Munich

The true founders of dermatology in Munich (setting aside the earliest developments under Horner) may be considered to be the following: Josef (von) Lindwurm (1824–74; he was, from 1863 onwards, professor regular), Karl Posselt (1837–1916) and Karl Kopp (1855–1912) who was head of the Reisingerianum policlinic. From 1913 to 1935, *Leopold Ritter von Zumbusch* (1874–1940) represented dermatology at the University of Munich; he was professor in ordinary from 1922 onwards. In 1910, a new policlinic was opened, and this was followed soon afterwards by a new complex of clinics in the Thalkirchner-Strasse. The name von Zumbusch is linked with the first accounts of 'Lichen albus' and of 'pustular psoriasis'. After von Zumbusch's retirement, *August Pöhlmann* (1882–1954), who had been his deputy, became his successor until 1937; he later became chief physician at the skin department at München-Schwabing. This department was opened in 1914 and was run at first by *Wilhelm Heuck*. From 1951 to 1974, *Curt Böhm*, a prominent proctologist, held this post, which is at present under *Hans-Jürgen Bandmann*.

After Pöhlmann's commission, *Julius Mayr* (1888–1965) became director of the Clinic of Dermatology of the University of Munich. He wrote a little handbook on artefacts; he was also popular with his students on account of his 'Kurzgefasstes Lehrbuch der Haut- und Geschlechtskrankheiten' (Concise Textbook of Skin and Venereal Diseases) published in 1929. Subsequently, the clinic was at first run provisionally by Heinrich Höcker and was then taken on by *Alfred Marchionini* (1889–1965). Marchionini's earlier fields of interest were the acid mantle of the skin surface (which he studied with Schade), the climatically influenced skin diseases, and dermatological ethnology. In 1950 he founded the periodical '*Der Hautarzt*', and instituted the publication of '*Ergänzungswerk*' zum Jadassohn'schen Handbuch der Haut- und Geschlechtskrankheiten (Supplementary volumes to Jadassohn's Handbook of Skin and Venereal Diseases') which up to now total 19 volumes. He managed to bring an international congress to Germany for the second time, but he did not live to see it taking place. His major significance lay in his co-founding a new internationality in dermatology. Being a much travelled ambassador of the subject he was convincingly successful. Marchionini's successor is *Otto Braun-Falco* (born 1922).

Since 1 October 1968, a professorship for skin and venereal diseases has been in existence at the Medical Faculty of the Munich Technical College; this is held by *Siegfried Borelli* (born 1924).

Bonn

As an antipode, as it were, to Breslau, the most important West German School of Dermatology developed in Bonn, in the Prussia of that time. *Joseph Doutrelepont* (1834–1918) had originally qualified in surgery and ophthalmology in Bonn in 1863. But soon afterwards he began to develop dermatology there and in 1894 he became honorary professor in ordinary; he later created his own Radiation Institute at his clinic. After having run a private clinic since 1879, Doutrelepont had placed at his disposal a clinic, which included a policlinic, with 60 beds. He was a self-taught dermatologist; he also tried his hand at experiments with carcinoma transplants and was, furthermore, one of the first to demonstrate Koch's tubercle bacillus in lupus vulgaris. Among his pupils were Fabry, Grouven, Heuck and Zieler. In 1910 he was succeeded by *Erich Hoffmann* (1868–59), whose participation in the discovery of the spirochaete of syphilis, when working in Berlin, had already earned him fame. After earlier work on syphilis of the veins and further venereological studies—such as the early diagnosis of syphilis by lymph node puncture—he developed his concept of the so-called maximum early treatment method. Some of his special descriptions include: perifolliculitis capitis abscedens et suffodiens; melanodermatitis toxica lichenoides et bullosa; naevus lipomatodes superficialis; generalized verrucosis; pyoderma chancriformis; and the first account to be written in German on dermatofibrosarcoma protuberans. His prominent colleagues included Friboes, Habermann, Memmesheimer, Rost, Rüete and Schreus. From 1934 to 1958, the director of the clinic was *Otto Grütz* (1886–63), who had qualified in Kiel in 1932. He had distinguished himself chiefly as a sound mycologist, and had also discovered, in association with Max Bürger, disordered fat metabolism in psoriasis. Grütz was succeeded by *Hans Schuermann* (1908–62) who had been a leading worker in Berlin and Würzburg, studying, among other topics, dermatomyositis and 'Diseases of the oral mucosa and lips'. (The latter now published under the co-authorship of Schuermann-Greither-Hornstein). Among his colleagues, the following are particularly well known: R. Doepfner (1920–64), W.Hauser (born 1913; acrodermatitis chronica atrophicans, lupus erythematosus, segmental relations in skin disease) and Hornstein (now of Erlangen). The present holder of the professorial chair at Bonn is Arthur Leinbrock (born 1908) who was appointed in 1964.

Göttingen

In Göttingen, consistent dermatovenereological work really began in 1917 with the appointment of *Erhardt Riecke* (1868–1942), at first as associate professor and from 1927 onwards as scheduled professor. His Handbook contribution on bullous diseases remains fundamental to this day as does the multi-author 'Lehrbuch der Haut- und Geschlechtskrankheiten' which he edited in 1909; a new edition of this book was produced by Bode and Korting in 1962 and again in 1970. In the same class are his '*Deutscher Dermatologenkalender*' (German Dermatologist's Diary) which appeared in 1929, and his '*Deutsches Dermatologenverzeichnis*' ('Index of German Dermatologists') published in 1939. In place of the latter book, '*Die Dermatologen deutscher Sprache*' ('Dermatologists of the German speaking area') edited by H.Löhe and E.Langer was published in 1955; its replacement (Riethe) is in preparation.

In Göttingen, Riecke was followed by *Walter Krantz* (1891–1970) who held the post from 1935 to 1945. From 1946 onwards, his earlier 'habilitant' *H.G.Bode* was in charge; he also was a leading figure at the Breslau clinic, and distinguished himself in basic work in roentgenology. He was the first to use beta rays (6 MeV Betatron) therapeutically in a skin clinic. Since 1974 H.Ippen has been head of the clinic.

Kiel

In Kiel, the general physician Heinrich Irenaeus Quincke (1842–1922) not only introduced the practice of lumbar puncture in 1891, but also described Achorion Quinckeanum, the acute oedema of the skin given his name in Germany; this was, in fact, reported in the first volume of the 'Monatshefte für praktische Dermatologie' in 1882, page 129. The first independent university dermatologist was *Ernst Dühring* (1858–1944) who took office in 1902. During his time as professor of dermatology at the Imperial College, Constantinople, he described an epidemic erythema multiforme exsudativum. Having been made a pasha he soon returned to Kiel to take charge of the famous 'White Stag' sanatorium in Dresden in 1906. His successor in Kiel was *Victor Klingmüller* (1870–1942), who earned international reputation especially as a competent leprologist and who also wrote exhaustively (partly with the co-operation of Dittrich) on the clinical picture of perniosis and erythrocyanosis. He was followed by *Josef Vonkennel* (1897–1963) who was, however, to go to Leipzig in 1943. In consequence, *P.W.Schmidt* took over, and remained from 1943–50, working particularly on mycology (Clinic for hyperkeratotic epidermophytosis) and on occupational dermatoses (baker's eczema). Since 1950, *Albin Proppe* (borne 1907) has been head of the clinic at Kiel.

Other University centres

Most of the other university dermatological clinics in the German Federal Republic became independent comparatively late. For example, in 1912, H.Hübner first became chief physician of the dermatological division of the medical clinic at *Marburg*. He was followed there by *Alfred Ruete* (1882–1951), who was, in 1924, able to take over a new building with 62 beds. The clinic was run by *K.W.Kalkoff* from 1950 to 1960; by *O.Braun-Falco* from 1961 to 1966; and by *C.G.Schirren* (superficial X-ray therapy) from 1968 to 1969. At present, *H.C.Friedrich* is head of the Marburg Clinic.

In *Giessen*, *Georg Sticker* described in 1899 '*Erythema infectiosum*' which E.Hoffmann later aptly named 'Ringelröteln' (*Ringed rubella*). In Jadassohn's Handbook there is 'Entwurf einer Geschichte der ansteckenden Geschlechtskrankheiten' penned by Sticker; this 'Draft of a history of infectious venereal diseases' is, even nowadays, worth reading. He read specialized dermatology in Giessen from 1901 to 1904 when he was followed, in 1905, by *Albert Jesionek* (1870–1935). At Giessen there also was, from 1913, the *first German sanatorium for lupus*; similar ones were subsequently provided at Möncheberg, Hornheide and Breslau. Then, in 1914, a new skin clinic was opened in Giessen, to be run by *R.M.Bohnstedt* (1907–70). *Leonhard Illig* (born in 1920) succeeded Bohnstedt at Giessen in 1970.

Erlangen's university was founded in 1743, and, although there were early signs of an emergent venereology there certainly at the beginning of the last century, it is only since the summer term of 1906 that regular lectures devoted exclusively to that subject have been given. They were originally delivered by *Leo Hauck* (1874–1945); his pupil, *F.Dietel* (born in 1897) took over the running of the largest municipal skin clinic in the German Federal Republic at Nurenberg. This clinic is at present under *G.Weber*, a former colleague of Keining and Korting of Mainz. For a while, the Erlangen clinic was under the directorship of *W.Richter*, who had also been successor to Marchionini at Ankara for a time. After Richter, C.H.Hasselmann held temporary office until the present chief, *Otto Hornstein*, took over.

Even in the oldest German university, at *Heidelberg*, where a few hesitant steps had been taken by, amongst others, R.A.Hecker, regular lectures on skin and venereal diseases did not begin until as late as the summer term of 1899, when they were delivered by *Siegfried Bettmann* (1869–1939). There the skin and medical clinics were eventually divorced in 1908, but Bettmann was not promoted to regular professor until 1922. This clinic at Heidelberg was run, from 1935 to 1956, by *Walther Schönfeld* (born in 1888) who is one of the grand old men of German dermatology. When he moved to Heidelberg from Greifswald, where he had been in charge of the town's university clinic, he took with him his enthusiasm for dye-therapy ('Greifswalder Farbstoffmischung). Among his published works are a voluminous dermato-venereological casuistry, several students' books such as 'Lehrbuch der Haut- und Geschlechtskrankheiten' in 1938, and the 'Atlas', which had Friboes as co-author, in 1949; but his history of German dermatology ('Kurze Geschichte der Dermatologie und Venerologie') was particularly illuminating. He and Gottron together published between 1958 and 1970 the five volume handbook '*Dermatologie und Venerologie*'. Schönfeld was succeeded by *Josef Hämel* (1894–1964), and then by *Urs Schnyder* (born in 1923).

In *Freiburg*, after a similar erratic start, dermatology became an independent specialty originally under *Eduard Jacobi* (1862–1915). He became well known not only for his two volume 'Atlas der Hautkrankheiten', which was published in 1904 and was the first dermatological atlas to be produced in four colour print, but also for his publishing, with Neisser, the 'Ikonographia Dermatologica', and also for his description of '*Poikiloderma atrophicans vasculare*', which nowadays is recognized as a consequence of dermatomyositis. Jacobi was succeeded by *Georg Alexander Rost* (1877–1970), who held the post until 1933. On account of his background in Naval medicine he was originally interested in venereology, but he later turned to radiotherapy and subsequently to allergology which led him to the study of 'Früh- und Spät-exudatives Eczematoid'. He had some particularly gifted pupils amongst whom Keller, who later went to Aachen, Marchionini, B.Ottenstein and *Alfred Stühmer* (1885–1957) can be counted. Stühmer was Rost's successor from 1934, having held the post as ordinary professor in Münster from 1925 to 1934. He was one of the first persistent proponents of Neosalvarsan. The Freiburg clinic had a period of transitory directorship under *Pfister*, the present chief physician at Karlsruhe, and was then taken over by *K.W.Kalkoff* (born in 1909).

The clinic at *Münster* in Westphalia was run from 1925 to 1934 by *Alfred Stühmer* (1887–1957) and subsequently from 1934 to 1937 by *J.K.Mayr* who was later to become professor at Munich. He was succeeded by *Carl Moncorps* (1896–1952), the originator of a clear system of classification of the keratoses, which was published in volume VIII, chapter 2, of Jadassohn's Handbook and still remains a valid one. He also carried out basic studies on the pharmacodynamics of ointments and the percutaneous absorption of medicaments from ointments. He was succeeded as director of the clinic, in 1953, by *Paul Jordan* (born in 1902); *Egon Macher* (born in 1924) has just been appointed there.

In *Tübingen*, *Paul Linser*, a graduate with Neisser in 1904, held his first course of lectures on dermato-venereology in 1905. Since 1920, at the same time of the year that Linser became personal professor, the skin and the ENT clinics have been in a building of their own. Linser remains celebrated in dermatological circles, not only on account of his famous pupils Memmesheimer and Vohwinkel, who worked on keratoma mutilans, but also as the father of modern sclerosing therapy for varicose veins. From 1936 to 1945, the clinic at Tübingen became the responsibility of *W.Engelhardt* (born in 1895), who was interested in mycology and sulphonamide pareses; his place in the skin clinic was taken and held until 1963 by *H.A.Gottron* (1890–1974), who had been the Breslau clinic's director between 1935 and 1945. Gottron was considered to be the most distinguished morphologist of his day. His name is linked with the clarification or new description of a whole set of clinical pictures including acrogeria and scleromyxoedema; in later years, however, he concerned himself particularly with the questions of carcinoma-sarcoma-reticuloses. He was a convinced general/pathologist ('Relationspathologie') and considered skin patients to have their own individual pathology determined by personal factors.

At *Breslau*, some of his pupils include Bode, Halter, who previously had been chief physician at Berlin-Spandau, Gertler (later also chief of Leipzig and of the Charité in Berlin) and *Nödl* who now is at the skin clinic of the University of *Homburg* on the Saar. To mention a few of his colleagues at *Tübingen*, there are: W.Schneider, W.Nikolowki, who now is chief physician at the Augsburg municipal skin clinic, G.W.Korting, R.Schmitz, H.C.Friedrich, W.Adam and Herbert Fischer. Gottron's handbook 'Dermatologie und Venerologie' written together with Schönfeld, has already been mentioned. Today the clinic at Tübingen is run by *W.Schneider* (born in 1910), who is also the present editor, together with *Tronnier*, of the periodical '*Berufsdermatosen*', which was founded by Schölzke in 1952.

Further university clinics emerged by degrees in West Germany from municipal origins; for example, in *Mainz* the fourteenth German university had been founded in 1477, the same year, in fact, as the universities were founded in Tübingen and Uppsala, but it had to be re-founded in 1946. Up till then there had been a municipal skin clinic under Hugo Müller, who was a proponent of bismuth therapy and J.Fischer. The first occupant of the professorial chair was Egon Keining (1892–1973); his principal pupils were Braun-Falco and G.Weber, and he was an eloquent, pragmatic clinician with clear views on guidelines to therapy. The clinic has, since 1961, been headed by G.W.Korting (born in 1919).

In the basin of the rivers Rhine and Main there lie three towns in a triangle, namely Mainz, Wiesbaden, where formerly Felke and now Grimmer work, and *Frankfurt*. This triplet of towns quickly won great prestige dermatologically through *Karl Herxheimer* who lived from 1861 to 1942 and directed not only the municipal but also the university skin and venerological clinics. Herxheimer became internationally famous probably mostly on account of the so-called Herxheimer's spirals, but also through the 'reaction' which was named in part after him (Jarisch, 1895; Herxheimer and Krause, 1902) and by virtue of the exhaustive description he and Kuno Hartmann made, in 1902, of acrodermatitis chronica atrophicans, which surpasses that of Pick and Buchwald.

After a break from 1930 to 1934, Herxheimer was followed from 1934 to 1945 by *Martin Schubert*, and, after a further break, by *Oscar Gans* (born in 1888) from 1949 to 1959. Gans had already by 1925 earned an early and lasting reputation as a histopathologist as a result of his writing a two-volume work 'Histologie der Hautkrankheiten'. His pupil G.K.Steigleder helped him to produce a second edition in 1955–56. Gans was succeeded, from 1962 to 1969, by his pupil *Franz Hermann* (born in 1898), and then by *Theodor Nasemann* (born in 1923).

Representing the specialty in *Köln* (Cologne) from 1919 to 1931 was *Ferdinand Zinsser* (1865–1952), who depicted in his 'Ikonographia Dermatologica' of 1906 the condition now known as dyskeratosis congenita. The name of his successor, *Friedrich Bering* (1878–1950), is probably best remembered by the younger generation of dermatologists by his monograph, written with Zitzke in 1935, entitled 'Die beruflichen Hautkrankheiten' ('The occupational dermatoses'). Josef Vonkennel (1897–1963) became chief of the clinic at Köln in 1950 after spending some time as a clinic director in Kiel and Leipzig. As milestones in his rich scientific career may be listed his studies on gold and arsenic, his work on chemotherapy, undertaken with Kimmig, using Globucid (Sulfaethidole) and Tecesal (Calcium theosulfate), and particularly his describing, with Lembke, Myocine and in 1944 penicillin. In 1964, *G.K.Steigleder* (born in 1925) followed in Vonkennel's footsteps.

In *Düsseldorf*, dermatology had been nurtured in its infancy by *Karl Stern* (1864–1935), and from 1923 it was practised in the medical Academy of the present-day university of Düsseldorf. Then, from 1930 to 1962, the clinic was run by *Hans Theodor Schreus* (1892–1970), who became well known principally because of his so-called *saturation therapy* with Salvarsan, and also on account of his being the first to use a sulphonamide in humans and his work on physical dosimetry of X-rays. He was, above all, technically gifted and developed an unique instrument for grinding skin. Since 1962, the clinic has been run by *Aloys Greither* (born in 1913).

The story of dermatology in *Hamburg* is, above all, a prime example of how, side by side with the evolution of dermatology within the university, the specialty has also been able to develop outside it to such an extent as to achieve world renown as a result of the individual efforts of eminent personalities. In the university, *Paul Mulzer* was chief from 1924 to 1945; there was then a brief interregnum by Marchionini from 1948 to 1950, after which *Josef Kimmig* (born in 1909) took the post which he still holds. There is no doubt that *Paul Gerson Unna* (1850–1929) was one of the great international founders of modern dermatology, and this he achieved while practising in a private dermatology clinic with its own laboratory and which offered a 'Dermatologicum' with regular teaching sessions. His name is primarily associated with the description of seborrhoeic eczema, of the ulerythemas (oophryogenes, sycosiforme and acneiforme) and of parakeratosis scutularis variegata; it is coupled with that of Thost in the description of keratoma palmare et plantare hereditarium. He established, for the study of lupus vulgaris, the technique of pressing the lesion with a glass slide—vitropression; this procedure had, however, been used two years previously by Oscar Liebreich, whom we also have to thank for lanolin. Unna introduced to the dermatological therapeutic repertoire such things as: ichthyol, resorcin, eucerin, zinc gelatin dressings (Unna's paste), adhesive plaster bandages, and comedo expressors. The following are a selection of his very numerous monographs: 'Sauerstofforte und Reduktionsorte', 'Biochemie der Haut', 'Histochemie der Haut', 'Internationaler Atlas von seltenen Hautkrankheiten' ('International Atlas of Rare Skin Diseases') which he produced with Morris-Duhring, and, especially, 'Histopathologie der Hautkrankheiten'. In 1882, he and *Hans von Hebra* founded the '*Monatshefte fur praktische Dermatologie*'. This publication came out, between volume 54 in 1912 and volume 154 in 1968, as '*Dermatologische Wochenschrift*', and it now appears as '*Dermatologische Monatsschrift*', which is the journal of the German Democratic Republic Dermatological Society. A further brilliant example, in Hamburg, of a representative of municipal dermatology is *Eduard Arning* (1855–1936), who published 'Über nodüli cutanei' (on skin nodules) and formulated a tincture which bears his name ('Arning's Tincture'). Moreover, there were in Hamburg, in 1939, five (!) dermatological departments being run in public hospitals. At the present time, the Hamburg university dermatology clinic is directed by *J.Kimmig*.

Municipal skin clinics

Limited space makes it impossible to deal here with other municipal skin clinics of the Federal Republic of Germany of today. However, an exception should be made in order to mention, as it is of historical interest, the *first private skin clinic in Germany*. This meritorious institution was founded in 1837 as a hospital for skin diseases (Heilanstalt für Flechtenkranke') and was situated in Bad Cannstadt near Stuttgart; it was run by the four members of the Veiel family who formed a dermatological dynasty there. One of them, Theodor Veiel, was the first German to introduce pyrogallol into the therapy of lupus vulgaris; he was, together with Herxheimer, Hammer and the Wiesbaden dermatologist Touton, also a co-founder of the *South West German Dermatological Association*, which still meets regularly. The important municipal skin clinic of *Stuttgart* nowadays is run by *W.Knoth*, that of *Bremen* by *Herzberg*.

German Democratic Republic

Likewise, after dealing already with the cradles of dermatology in Berlin and Breslau as they are historically important, insufficient space is left to permit a detailed account of the evolution of dermatology in the universities and large towns of the *German Democratic Republic*. This is, however, such an important element in the overall development of German dermatology, that the specialty in centres such as Greifswald, Halle, Jena, Leipzig and Rostock justify a separate article.

CONCLUSION

It is to be hoped that this necessarily superficial and incomplete synopsis may convey to the reader the impression that, right up to the present day, German dermatology has lacked neither individuals nor schools of such a calibre that it deserves the highest regard in the framework of international dermatology.

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BIBLIOGRAPHY

Buley, Hans M. (1952) German Schools of Dermatology in the Past Century. *Archives of Dermatology and Syphilis*, **66**, 440.

Burgun, Rene Strasbourg sa Contribution à la Dermatologie.

Diepgen, Paul (1960) *Unvollendete. Vom Leben und Wirken frühverstorbener Forscher und Ärzte aus anderthalb Jahrhunderten*, pp. 126–141. Georg Thieme Verlag, Stuttgart.

Eulner, Hans-Heinz (1970) *Die Entwicklung der medizinischen Spezialfächer an den Universitäten des deutschen Sprachgebietes*. Ferdinand Ende Verlag, Stuttgart.

Gertler, W. (1973) *Systematische Dermatologie und Grenzgebiete*. Bd. III, 2.32 ff. VEB Georg Thieme, Leipzig.

Gottron, H.A. & Walther, H. (1954) In memoriam Leo Hauck. *Zeitschrift für Haut-und Geschlechtskrankheiten*, **25**, 318.

Gottwald, Werner (1968) Zur Geschichte der Universitätshautklinik in Breslau. *Medizinische Welt*, **19**, 346–350; 403–412.

Greither, A. (1964) Die Dermatologen-Dynastie der Veiel. *Hautarzt*, **15**, 197–201.

Hebra, Ferdinand (1872) Scabies, Krätze. *Lehrbuch der Hautkrankheiten*. I. Bd. S. 495–560.

Hoffmann, E. (1948) *Lebenserinnerungen aus einer Wendezeit der Heilkunde*. Schmorl u von Seefeld Nachfolger, Hannover.

Hollander, Alfred (1950) Scientific work of Paul Unna. In commemoration of the one hundredth anniversary of his birth. *Archives of Dermatology and Syphilology (Chicago)*, **62**, 351.

Hollander, Alfred (1955) Fiftieth anniversary of the discovery of the spirochaeta pallida. *Archives of Dermatology and Syphilology (Chicago)*, **71**, 289.

Hollander, Alfred (1960) In memoriam Erich Hoffmann (1868–1959) *Proceedings of the Rudolf Virchow Medical Society in the City of New York*. Vol. XIX. S. Karger, Basel.

Hollander, Alfred (1968) Paul Gerson Unna: 1850–1929. *Dermatology Digest*, 47.

Kalkoff, K.W. (1973) Geschichte der Dermatologie. Die ersten Tuberkuloseheilungen durch Conteben—25 Jahre danach. *Hautarzt*, **24**, 546.

Korting, G.W. (1962) Egon Keining zum 70. Geburtstag. *Dermatologische Wochenschrift*, **146**, 530.

Korting, G.W. (1962) Hans Schuermann, 1908–1962. *Hautarzt*, **13**, 562.

Korting, G.W. (1964) H.-G. Bode—sexagenarius. *Zeitschrift für Haut und Geschlechtskrankheiten*, **37**, 139.

Korting, G.W. (1965) Biographische Notizen über Alfred Buchwald. *Hautarzt*, **10**, 456.

Korting, G.W. (1970) H.A.Gottron—octogesimus. *Hautarzt*, **21**, 145.

Korting, G.W. & Schneider, S. (1972) Zur 50. Wiederkehr des Todestages von Frau Marie Kaufmann-Wolf am 1.3.1972. *Hautarzt*, **23**, 362.

Korting, G.W. (1974) Heinrich Adolf Gottron zum Gedenken. *Deutsche medizinische Wochenschrift*, **39**, 1940.

Mulzer, P. (1936) Eduard Arning. *Dermatologische Wochenschrift*, **41**, 1385.

Nave, Ilse (1951) *Zur Geschichte des Lehrstuhls für Dermatologie und Syphilis an der Universität Breslau*. Inaugural Dissertation.

Proppe, Albin (1969) Das wissenschaftliche Werk von Hans Theodor Schreus. *Jahrbuch der Universität Düsseldorf*, 17–25.

Roederer, Jean *Cinq Semestres A La Faculte De Medicine De Strasbourg*. Souvenirs d'un étudiant alsacien Annuaire de la Societé des amis des Vieux Strasbourgs.

Rost, G.A. (1953) Semuel Hafenreffer, Autor des ersten Lehrbuches der Dermatologie im deutschen Kulturraum. *Zeitschrift für Haut- und Geschlechtskrankheiten*, **14**, 227.

Rost, G.A. (1957) Das Ei als seltenes Attribut eines Arztes. *Sudhoffs Archiv für Geschichte der Medizin und der Naturwissenschaften*, **41**, 255.

Sönnichsen, N. (1972) Fritz Richard Schaudinn zum 100. Geburtstag. *Dermatologische Wochenschrift*, **158**, 73.

Schmitz, Sigrid Albert Neisser. Leben und Werk auf Grund neuer, unveröffentlicher Quellen. *Düsseldorfer Arbeiten zur Geschichte der Medizin*, Vol. 29. Michael Triltsch Verlag, Düsseldorf.

SCHÖNFELD, W. (1938) Friedrich Wilhelm Felix von Baerensprung (1822–1864). *Archives of Dermatology and Syphilology (Berlin)*, **176**, 579.

SCHÖNFELD, WALTHER (1954) *Kurze Geschichte der Dermatologie und Venerologie und ihre kulturgeschichtliche Spiegelung*. Theodor Oppermann Verlag, Hannover-Kirchrode.

SCHÖNFELD, WALTHER (1957) Das Alter von 'Lupe' und 'Diaskopie' in der dermatologischen Diagnostik. *Dermatologische Wochenschrift*, **136**, 1231.

SCHÖNFELD, WALTHER (1946) Erlebte Geschichte der Venerologie und Dermatologie. *Hautarzt*, **15**, 245.

SCHÖNLEIN, J.L. (1846) *Allgemeine und spezielle Pathologie und Therapie nach dessen Vorlesung niedergeschrieben und herausgegeben von einigen seiner Zuhörer*, S.41.

SCHWENINGER, E. & BUZZI, S. (1891) Multiple benigne geschwulstartige Bildungen der Haut. *International Atlas seltener Hautkrankheiten*, Heft V. Voss, Hamburg und Leipzig.

STÜRZBACHER, M. (1959) Zur Geschichte der Dermatologie in der Berliner Charité. *Zeitschrift für Haut- und Geschlechtskrankheiten (Berlin)*, **26**, 319.

WEINERT, CORNELIA (1970) *Die Geschichte der Dermatologie und Venerologie*. Inaugural Dissertation, München.

WERNSDÖRFER, R. & DIETEL F. (1969) Die Universitäts-Hautklinik Erlangen bis 1945. *Hautarzt*, **20**, 518.

WICHMANN, J.E. (1791) *Beytrag zur Kenntnis des Pemphigus*. G.W.Keyser, Erfurt.

WICHMANN, J.E. (1794) *Ideen zur Diagnostik*. Hannover, in der Helwingschen Hofbuchhandlung.

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Brief Communication

A rapid method for identifying nucleated horn cells

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Fluorescent microscopy has been widely used in the biological field but only occasionally as an adjunct to the usual methods applied to skin biopsies (Jarrett, Bligh & Hardy, 1956). Similarly, although cytological examination is almost routinely carried out in several medical specialities, it is not regularly performed in examinations of skin.

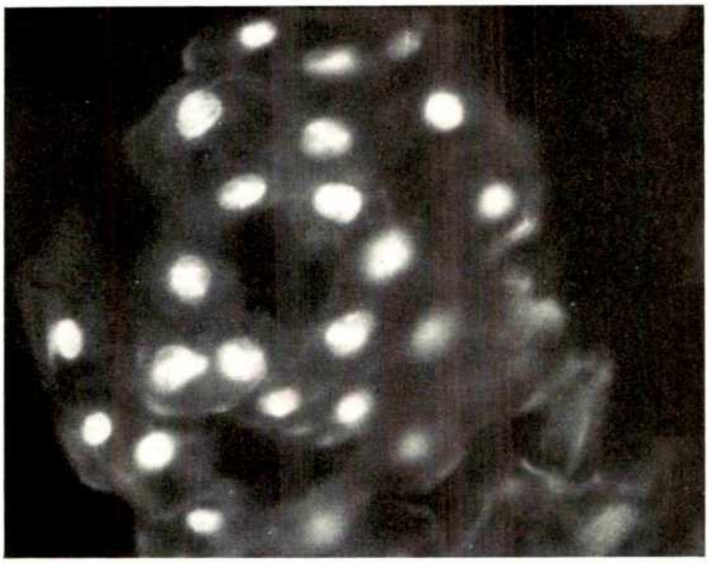

FIGURE 1. Micrograph of parakeratotic stratum corneum from psoriatic plaque stained with diamidinodiphenylamine dihydrochloride for approx. 1 min. (Magnification × 180.)

Goldschmidt & Thew (1972) published an analysis of cytological abnormalities which they found in horn cells taken from a large series of patients with orthokeratotic and parakeratotic disease (i.e. psoriasis, seborrhoeic dermatitis and various types of dermatitis–eczema). A modified Giemsa stain technique was used to identify nuclei in horn cells obtained by several sampling methods. Significant differences between psoriasis and most other dermatoses in the frequency of halo and nucleated horn cells were reported. However, the method had a serious disadvantage for routine and extensive use because of the long time necessary to obtain reliable results with a quixotic stain.

Tring & Murgatroyd (1976) have recently described the convenience and extremely rapid action of a fluorescent stain, diamidinodiphenylamine dihydrochloride. This compound was one of several aromatic diamidines deliberately incorporated into synthetic media to suppress contaminants of *H. pertussis* cultures (Lacey, 1951; Nicholson & Turner, 1954). A fortuitous observation that diamidinodiphenylamine dihydrochloride selectively stained DNA prompted its use as a stain to identify nuclei and DNA residues in horn cells.

Adhesive slides using double coated cellophane tape can be used to strip samples of horn cells. The cell coated slide is immersed in a Coplin jar containing a 0·2% solution of diamidinodiphenylamine dihydrochloride for $\frac{1}{2}$–1 min. After washing under tap water for 2–3 min the sample is examined under a fluorescent microscope equipped with suitable filters to provide ultraviolet excitation. Nuclei and nuclear particles brilliantly fluoresce (Fig. 1) and can be rapidly identified.

This method is currently being used to evaluate various topical applications and their ability to modify parakeratosis. Preliminary experiments also indicate that the method can be used to identify rapidly certain cutaneous fungal infections.

REFERENCES

GOLDSCHMIDT, H. & THEW, M.A. (1972) Exfoliative cytology of psoriasis and other common dermatoses. *Archives of Dermatology*, **106**, 476.

JARRETT, A., BLIGH, A. & HARDY, J.A. (1956) Fluorescent microscopy of the human skin. *British Journal of Dermatology*, **68**, 111.

LACEY, B.W. (1951) Selective media for *H. pertussis* and *H. parapertussis*. *Journal of General Microbiology*, **5**, VI.

NICHOLSON, D.E. & TURNER, G.C. (1954) A selective medium for the primary isolation of *H. pertussis* and *H. parapertussis*. *Journal of General Microbiology*, **11**, 91.

TRING, F.C. & MURGATROYD, L.B. (1976) A new fluorescent stain for cell nuclei. Stain Technology (in press).

British Journal of Dermatology (1976) **94**, 341.

Comment Article

LIPOID PROTEINOSIS: URBACH–WIETHE DISEASE

The student of a rare disease is often at a disadvantage in that he has to base his conclusions on personal experience of a very limited number of cases, supplemented by such information as he can derive from case reports which may fail to describe in sufficient detail, or even to mention, those aspects of the disease which seem to him to be of particular importance. Lipoid proteinosis must be regarded as rare as only about 200 cases have been reported, since Urbach & Wiethe (1929) gave the first clear description of the disease over 45 years ago. Dr P.-Å. Hofer, of the Department of Pathology of the University of Umeå, in northern Sweden, has taken full advantage of his opportunity to study twenty-seven cases in fourteen families. His publications, which together provide a really comprehensive account of the disease, have appeared in many different journals, the variety of which emphasizes the interest of the condition to specialists in such diverse disciplines as genetics, pathology, general medicine, dermatology, otolaryngology, and stomatology. The purpose of this short review is to draw attention to the valuable contributions of Dr Hofer and his colleagues, and to summarize their main findings and conclusions.

Although there are obvious objections to the use of eponyms, notably the fact that their usage is frequently not internationally uniform—Graves' disease becomes Basedow's disease on crossing the English Channel—Hofer (1973) considers the term Urbach–Wiethe Disease (UWD) preferable to lipoidosis cutis, Urbach & Wiethe's original suggestion, or lipoid proteinosis or hyalinosis cutis, which some subsequent authors have preferred. Such terms, vague though they are, imply a knowledge of the basic biochemical defect, which we do not possess.

A genetic investigation of the Swedish patients (Hofer, 1974b) confirmed that UWD is apparently transmitted by an autosomal recessive gene. Only two authors have reported possible dominant inheritance. Seventeen of the eighteen living parents of the Swedish patients were examined as were seventeen of their twenty-nine living children. Heterozygotes showed some of the cutaneous or mucosal manifestations of UWD, but in mild degree. Most of the over 200 reported cases have been of European descent and about a fifth of them have been the South African descendants of a German immigrant (Heyl, 1970). Hofer thinks it not impossible that the South African and the Swedish patients have common ancestors. Diabetes occurred in the first degree relatives of patients in four of the fourteen families and in other relatives in four additional families (Hofer *et al.*, 1974).

The classical lesions of UWD involve the skin and the oral, pharyngeal and laryngeal mucous membranes, but the disease is often much more extensive. Hoarseness is usually present from infancy or early childhood, and was a feature of twenty-six of the twenty-seven Swedish cases (Hofer & Öhman, 1974): it results from incomplete closure of the vocal cords, which are thickened and irregular. Although in most cases skin lesions appear at the same time or shortly after the hoarseness, they may precede the latter by several years, and in exceptional cases there may be no clinical manifestations until after puberty.

Oral lesions were present in all the Swedish patients, and tended to increase in severity and extent with age (Hofer & Bergenholtz, 1975). In young patients areas of induration were limited to the lip mucosa and to the back of the tongue. By the second decade there were granular lesions of the lips, and papules on the tongue and palate. The affected mucosae then became progressively paler and showed some pitting. Induration of the frenulum reduced mobility of the tongue. Later increased induration, fissuring and scarring were prominent. In all patients the posterior wall of the pharynx showed patches or a network of white or yellowish lines. The teeth tended to be lost early.

Lesions resembling acne scars are of course the typical cutaneous change, and are not confined to the face; they are seen also on forearms and lower legs. However these are a feature of the second stage of the disease. In the first stage which may continue into adolescence or early adult life, there are haemorrhagic crusts, crops of bullae, and pustules. The first and second stages overlap to a varying degree, and it is not certain that all 'scars' are preceded by crusts. Nodular or diffuse areas of infiltration appear on the face, and to some extent in the axillae and on the scrotum; beaded papules on the eyelid margins are distinctive, but inconstant. In the scalp, and in the male beard, such infiltrations may cause patches of alopecia. Verrucous hyperkeratosis forms over areas exposed to trauma, such as the fingers, elbows, knees and buttocks. In some patients, however, the skin lesions may be so inconspicuous that they are easily overlooked.

The frequency of involvement of other organs is difficult to determine precisely as only three patients have been submitted to complete autopsy, but in some cases there have been lesions of the trachea and bronchi, of the upper and perhaps also the lower gastro-intestinal tract, and of the central nervous system. These latter are the most frequent for pathognomonic intracranial calcification was found in fifty-two of 100 cases in which X-ray examination of the skull was carried out; a bilateral sickle shaped area of calcification was found lateral to and just above the dorsum sellae. The calcification is intracerebral, and is sometimes responsible for epilepsy and a variety of hallucinatory symptoms.

Light and electron microscopy, and histochemical studies are fully reported (Hofer, 1973; Hofer *et al.*, 1974), but 'have not provided any final solution to the pathogenetic problems of the disease' (Hofer, 1974a), nor have they provided support for any of the published hypotheses.

The careful reading of Hofer's excellently illustrated papers forces one to endorse his speculation that the apparently greater rarity of the disease in some countries could be due to lack of familiarity with its manifestations.

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REFERENCES

HEYL, T. (1970) Genealogical study of lipoid proteinosis in South Africa. *British Journal of Dermatology*, **83**, 338.

HOFER, P.-Å. (1973) Urbach-Wiethe Disease (lipoglycoproteinosis; lipoid proteinosis; hyalinosis cutis et mucosae). *Acta dermato-venereologica*, **53**, Supplementum 71.

HOFER, P.-Å. (1974a) Urbach-Wiethe Disease. *Umeå University Medical Dissertations* No. 19, (Swedish, with a long summary in English.)

HOFER, P.-Å. (1974b) Urbach-Wiethe Disease (lipoglycoproteinosis; lipoid proteinosis; hyalinosis cutis et mucosae). *Hereditas*, **77**, 209.

HOFER, P.-Å. & BERGENHOLTZ, A. (1975) Oral manifestations in Urbach-Wiethe Disease (lipoglycoproteinosis; lipoid proteinosis; hyalinosis cutis et mucosae). *Odontologisk Revy*, **26**, 39.

HOFER, P.-Å., LARSSON, P.-Å., EK, B., GÖLLER, H., LAURELL, H. & LORENTZON, R. (1974) A clinical and histopathological study of twenty-seven cases of Urbach-Wiethe Disease. Dermatologic, gastroenterologic, neurophysiologic, ophthalmologic, and roentgen diagnostic aspects, as well as the results of some clinicochemical and histochemical examinations. *Acta Pathologica et Microbiologica Scandinavica*, Section A, Supplement No. 245.

HOFER, P.-Å. & ÖHMAN, J. (1974) Laryngeal lesions in Urbach-Wiethe Disease (lipoglycoproteinosis; lipoid proteinosis; hyalinosis cutis et mucosae). *Acta Pathologica et Microbiologica Scandinavica*, Section A, **82**, 547.

URBACH, E. & WIETHE, C. (1929) Lipoidosis cutis et mucosae. *Virchows Archiv für pathologische Anatomie und Physiologie*, **273**, 285.

British Journal of Dermatology (1976) **94**, 343.

Correspondence

DERMATITIS HERPETIFORMIS AND IODINE

SIR, Recently, Douglas & Alexander (1975) reported on dermatitis herpetiformis and its relationship to iodine and thyroid disease. I wish to describe briefly a case which may also be of interest. Mrs C.A., a 65-year-old female, had carried the diagnosis of dermatitis herpetiformis for 15 years. She had been unable to eat seafood for at least 12 years because of an expected cutaneous flare in her dermatitis herpetiformis (D.H.) on the following day. She had been well controlled with dapsone 100 mg a day, having mild pruritus only occasionally. She was seen by me in the autumn of 1974 when she was found to have sprue-like small bowel biopsy, confirmatory skin biopsies, direct immunofluorescence, and a severe flare of a classical D.H. eruption 4 days after stopping dapsone. She was placed on a gluten free diet and after a period of 6 months was able to stop her dapsone completely.

On her final revisit she remarked that she was now able to eat seafood without any cutaneous difficulty. To my knowledge she had no evidence of thyroid disease. Could this represent a relationship involving gluten, iodine, a sprue bowel, or the thyroid gland? Unfortunately she is lost to follow up.

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REFERENCE

DOUGLAS, W. S. & ALEXANDER, J. O. D. (1975) Dermatitis herpetiformis, iodine compounds and thyrotoxicosis. *British Journal of Dermatology*, **92**, 599.

A CASE OF PSEUDOXANTHOMATOUS MASTOCYTOSIS

SIR, Griffiths & Daneshbod (1975) reported an Iranian patient with pseudoxanthomatous mastocytosis. We, too, have found a case of this rare variety of urticaria pigmentosa.

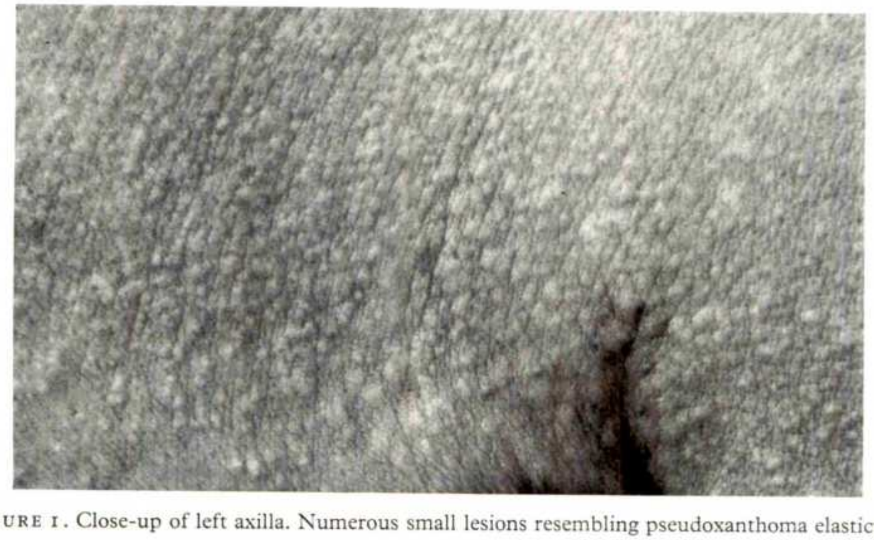

FIGURE 1. Close-up of left axilla. Numerous small lesions resembling pseudoxanthoma elasticum.

A Finnish male aged 83 years had coronary heart disease; otherwise he had been healthy. The family history did not reveal any skin diseases. For at least 15 years the patient had noticed soft, flat, yellowish nodules (from 1 to 10 mm in diameter) on his neck, around the axillae and in the antecubital areas (Fig. 1). The nodules were associated with slight intermittent itching. The lesions did not urticate on friction.

On the basis of the clinical picture pseudoxanthoma elasticum was suspected. However, no angioid streaks were seen in his retina. Routine laboratory tests (including serum cholesterol and triglycerides) showed normal values. X-rays of the skull showed one well demarcated radiolucent lesion (10 mm in diameter) on the occipital border of the right parietal bone. The lesion might have been a mastocytoma. X-rays of the bones of the extremities showed small radiolucent lesions which were probably due to osteoporosis.

A skin biopsy from the left axilla showed a moderate, patchy round-cell infiltrate in the dermis. Toluidine blue staining showed a markedly increased number of mast cells in this infiltrate. The elastic fibres were normal. Lipid stains revealed no fat in the lesions.

The clinical picture of this patient was very similar to that of the case published by Griffiths & Daneshbod, suggesting pseudoxanthoma elasticum. The correct diagnosis can easily be made by detecting increased numbers of mast cells in skin biopsies with normal elastic fibers.

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REFERENCE

Griffiths, W.A.D. & Daneshbod, K. (1975) Pseudoxanthomatous mastocytosis. *British Journal of Dermatology*, **93**, 91.

CYCLOPHOSPHAMIDE AND PEMPHIGUS

Sir, Dr Pasricha and his colleagues (1975) have confirmed that it is inadvisable to attempt to control severe pemphigus vulgaris in the initial stages with cyclophosphamide alone. They have not, however, convinced me of the truth of their main conclusion that cyclophosphamide is ineffective during the acute stages of pemphigus, since they used the drug for only 20 days. My impression is that immunosuppressants may take a month or more for their effects on pemphigus to become clinically obvious, and it is wrong to conclude after 3 weeks that the drug is having no effect.

It has been shown that in the treatment of systemic lupus erythematosus, cyclophosphamide takes 4–12 weeks to produce clinical improvement (Hadidi, 1970), and in rheumatoid arthritis the maximum benefit may not be seen for at least 6 months (Fosdick, Parsons & Hill, 1968).

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REFERENCES

Fosdick, W.M., Parsons, J.L. & Hill, D.F. (1968) Long-term cyclophosphamide therapy in rheumatoid arthritis. *Arthritis and Rheumatism*, **11**, 151.

Hadidi, T. (1970) Cyclophosphamide in systemic lupus erythematosus. *Annals of Rheumatic Disease*, **29**, 673.

Pasricha, J.S., Sood, V.D. & Minocha, Y. (1975) Treatment of pemphigus with cyclophosphamide. *British Journal of Dermatology*, **93**, 573.

British Journal of Dermatology (1976) **94**, 345.

Thesis

Chronic Urticaria. Thesis presented by H.M.G.DOEGLAS (1975) Groningen, The Netherlands, pp. 86.

This thesis describes the author's very extensive investigations of 141 cases of chronic urticaria. By careful investigation he was able to demonstrate one of the physical urticarias, at least as one component, in 55% of all cases, a much higher incidence than is usually reported. The considerable overlap between the various types of urticaria is confirmed. A background of atopy was not more common in most types of urticaria, with the notable exception of dermographism for which a role for IgE has recently been proposed. No useful information was obtained from routine allergy skin tests. A search for and eradication of focal sepsis were of no definite value but should not be abandoned. Three patients may have been helped by treatment of a worm infestation but a blood eosinophilia, which was sometimes found, could not be relied upon as a screening test.

Dr Doeglas confirms the value of a diet free of salicylates, benzoates and azo dyes in several patients, and also the curious empirical treatment of cold urticaria with penicillin injections.

Four of the chapters have already been published: in the *British Journal of Dermatology* (**93**, 135); in the *Archives of Dermatology* (**110**, 382; **111**, 979); and in the *Journal of Medical Genetics* (**11**, 31). In these papers it has been shown that test doses of salicylates and of azo dyes cause exacerbation not only of chronic idiopathic urticaria but even more often of cholinergic urticaria and of the variety that may be associated with pressure urticaria.

As hereditary angio-oedema has been shown to be caused by a deficiency of an inhibitor, a study was made of protease inhibitors in the serum of patients with other types of chronic urticaria. In general the levels were normal but $\alpha 1$ antitrypsin and antichymotrypsin inhibitor were found to be decreased in acquired angio-oedema and in acquired cold urticaria.

Book Reviews

Annual Review of Allergy (1974) Edited by CLAUDE A.FRAZIER (1975) Flushing, N.Y.: Medical Examination Publishing Co. Inc. Pp. 442. Price £7.50.

This is the third edition of *Annual Review of Allergy* edited by the energetic Dr Frazier. Published in July 1975, it covers the literature for 1973 in 31 chapters. The contributors are nearly all American, mostly but not all being allergists. Most of the articles include a general review of their topic and then individual papers are itemized in a style between that of the Year Book and of Excerpta Medica.

Despite the very wide ranging interests of American allergists (which may at times alarm us but which also provides us with clinical surprises), there is much in this volume relevant to dermatology. The chapter by Philip Blank on Immunodermatology alone contains 220 references, many from the familiar dermatology literature but many also from the less familiar allergy literature.

D. Burrows, the only contributor from the UK, discusses the interesting problems of the objective assessment of occupational disability. There are chapters on vasculitis, auto-immune disease, immunotherapy, and allergy to arthropods.

As with the Year Book and Excerpta Medica, this is a relatively painless way of catching up with missed ideas and references which may or may not here be critically evaluated. There are few illustrations, many tables, and even more spelling mistakes.

R.H.CHAMPION

Delayed Hypersensitivity. JOHN L. TURK, 2nd Revised Edition (1975). Amsterdam & Oxford: North-Holland Distributing Co. Pp. 313. Price £16.40

In the past decade immunology has been a major growth field, and among the ever expanding array of immunological journals, reviews and monographs it can be very difficult for the non-specialist to sort the wheat from the chaff. In the general interest weekly journals immunological topics are also popular, and this can at times be a problem for those who learned as medical students that lymphocytes could be small, medium or large, but had no clear cut function.

The second edition of John Turk's monograph of Delayed Hypersensitivity provides a reliable method of updating one's immunological understanding; major advances in knowledge of cell mediated immunity occurring up to 1973 are discussed with clarity. It is evenly divided between morphological and functional aspects of delayed hypersensitivity, beginning with a description of the mechanism of production of delayed hypersensitivity, and continuing with an equally concise description of the histology of delayed hypersensitivity reactions. Passive transfer and the homograft reaction are dealt with in detail, and there is an outstandingly clear explanation of immunological unresponsiveness. A further section is devoted to detailed consideration of lymph node histology and its alteration during the induction of delayed hypersensitivity and this is followed by discussions of antigen specificity and the role of macrophages. Consideration of the correlation between delayed hypersensitivity and cellular immunity, and a discussion of experimental models of auto-immune disease and possible clinical counterparts conclude the book.

This is not a book which many dermatologists will want to read regularly in its entirety. It is more likely to find a place on the bookshelf as an invaluable reference source, and perusal of individual chapters in relation to specific clinical problems will increase the understanding of pathological processes involved in many conditions.

Highlights for me were mainly the clear cut summaries of the author's own work; and explanation of the Jones Mote reaction and its modifications by cyclophosphamide, and balanced accounts of passive transfer of delayed hypersensitivity in both animals and a man. The mechanisms underlying tolerance and the section on *in vivo* and *in vitro* correlation of reactions in leprosy are exceptionally clear. There are few areas which leave one wanting further information, but the section on lymphokines is perhaps rather brief, and as a dermatologist I would have welcomed more consideration of allergic contact dermatitis.

This is to be recommended for all library bookshelves as a reference work, and for the personal bookshelves of those whose interest in immunology is deeper than a knowledge of Gell & Coomb's classification of immunological reactions. Regrettably it will rapidly require revision and updating to keep pace with the current spate of immunological literature.

RONA M. MACKIE

Clinical Tropical Dermatology. Edited by ORLANDO CANIZARES (1975) Oxford: Blackwell Scientific Publications. Pp. 464, illustrated colour slides. Price £20.00.

This new book of 464 pages, of which nearly 100 are devoted to dermatoses due to animals, is excellent and stimulating. So complete is the animal section that injuries by alligators and crocodiles receive attention, which surely widens the scope of dermatology. To the English dermatologist suffering from a diet of warts, eczema, psoriasis and the side effects of steroids and politics, the catalogue of exciting knowledge provokes urgent emigratory symptoms.

A team of sixteen experts of international reputation from the New World, India, Philippines, Mozambique, Israel and England, has combined with Dr Canizares to produce a nicely sized volume, which is intended primarily as a practical manual for the management of skin disease in the tropics, and is also designed to be of use to physicians who meet these dermatoses in temperate climates.

Tropical dermatology is an elusive thing to define, and the content of the book is sensibly separated into detailed accounts of the dermatoses which are prevalent in the tropics, and the special features of the more cosmopolitan ones when they are encountered in the tropics.

The volume itself is of the usual Blackwell high standard, with the print and layout familiar to all readers of *Textbook of Dermatology*, edited by Rook, Wilkinson & Ebling. Tables of differential diagnoses, clinical features of related diseases, characteristics of different drug reactions, etc. are also reminiscent of the *Textbook*, together with a section on topographical diagnosis.

The large group of colour photographs is mostly of high standard, but it is a pity that the text does not refer to the complaints so illustrated. An appendix giving a simple formulary for topical therapy, which contains no proprietory items, is of real value to the dermatologist practising in the tropics, which so often implies underdevelopment and underprivilege. But it is surprising to see ammoniated mercury ointment still advocated. In view of the importance of animal vectors of disease in the tropics, a second appendix of diseases transmitted from animal to man is a valuable and comprehensive source of information.

Criticisms are few, but the most maddening feature of the book is the system of 'selected references' or no references. These are at the ends of groups of chapters, instead of easily found after each description of disease with its treatment. Where a brief reference is cited in the text, it may or may not appear complete under the relevant 'selected references' heading. An example is to the subject Tanapox (which is also missing from the index), where Downie *et al.* (1971) is cited. After a long hunt for the relevant 'selected references' section one reaches the conclusion that this one has not been selected. But the author of 'plant dermatitis in the tropics' is not easily caught out. All his references are perfect, and well classified, and you know you are in the hands of the master.

Misprints are not too numerous, and as the quality of photographs and line drawings is in general excellent, one can forgive a little camera-shake in the photograph of bubonic plague (black death).

The rise in price of the volume from about £10 quoted in March to £15 in June, to the present £20 is frightening and requires justification.

R.R.M.HARMAN

Erythropoietic Protoporphyria. H. BAART DE LA FAILLE (1975) Utrecht: Oosthoek, Scheltema & Holkema. Pp. 165, illustrated. Price Dfl.75.

This thesis is the result of a study over a number of years of a group of subjects suffering from erythropoietic protoporphyria and deals with the basic clinical and photobiological features, along with the results of routine haematological and biochemical tests. Although this forms only the background to the main part of the thesis, it contains evidence of the author's detailed knowledge of this form of porphyria, and although not demonstrating new facts, it is of value as the product of a somewhat unique clinical experience. It is to be regretted that the detailed photobiological features were not included but are to form a separate publication.

The remainder of the study is taken up with an evaluation of the results of histopathological, histochemical, immunohistochemical and electron microscopic techniques applied to biopsy material following irradiation of the skin. Detailed descriptions are given of the changes occurring in dermal blood vessels and cells, and also in the epidermis, and these findings are used to try to explain the pathogenesis of the changes seen in the skin in erythropoietic protoporphyria.

This is a valuable contribution to the problem of erythropoietic protoporphyria.

W.FRAIN-BELL

Skin Deep. The Mystery of Tattooing. RONALD SCUTT and CHRISTOPHER GOTCH (1974) London: Peter Davies. Pp. 205, illustrated. Price £8.50.

Surgeon-Captain Ronald Scutt, now retired from the Royal Navy though still in active dermatological practice, is renowned for his knowledge and interest in tattooing. In the introduction to *Skin Deep*, he writes 'Due to the esoteric nature of the subject there has been a paucity of comprehensive books on tattooing, designed for general consumption . . .' Christopher Gotch, his co-author, shares his fascination for the subject and has collaborated in writing this absorbing, engagingly written and lavishly illustrated book. It comprises fifteen chapters, two appendices, including Who's Who in the Tattoo World, an extensive bibliography and index to match. All aspects of tattooing, historical, artistic, technical, social, sexual and medical are covered with the insight and authority of personal research and experience. The do-it-yourself dermatologist will not be disappointed; he can refresh his memory on the technicalities of the chemical removal of tattoos.

One criticism, and possibly a personal one of the reviewer, is that the 'Tattooing of Minors Act' which became law in 1969, receives only a footnote. The passage of this Act entailed lengthy debates in both Houses of Parliament and deserves more than a passing mention.

Though not a book for family viewing, *Skin Deep* is excellent value for £8.50 and can be recommended without reservation.

PATRICK HALL-SMITH

British Journal of Dermatology (1976) **94**, 349.

Review Article

The medical and social fate of the dichromate allergic patient

Despite the fact that allergic contact dermatitis from medicaments plays the leading part in the drama of the whole variety of allergic skin reactions (Bandmann & Breit, 1974), dichromate ranks number one of all contact allergens for male patients (Fregert *et al.*, 1969; Fregert, 1975). Of all positive reactions 7·6% are due to an allergy against dichromate, increasing to 10·7 or 11·2% for male patients (Bandmann & Breit, 1974; Fregert *et al.*, 1969). Details of the dichromate sensitivity were reported by Cronin in 1971. Already the preponderance of the male sex pointed in the direction of an occupational influence on this disease. It could be shown that cement workers, masons, painters, drilling-machine workers and galvanizers had the highest risk for becoming sensitized by dichromate (Eberhartinger & Ebner, 1968).

Kühl (1971), investigating masons and construction workers, found that eczemas from dichromate can develop in all age groups. Høvding (1970) stated that the development of cement eczema in older age groups seems to be a characteristic feature of this disease. Because dichromate is not a very potent allergen (Kligman, 1966) a considerably long time of exposure is required. In the literature there are various data for the time necessary to develop a sensitivity, ranging from 3·7 years (Wagner & Bredahl, 1965) to 23·8 years (Türk, 1975) for masons and construction workers, and from 17·0 years (Salfeld, 1968) to 20·4 years (Türk, 1975) for painters. Without taking into account the individual occupation Türk (1975) reported an average time of 20 years from starting the occupation to the outbreak of the skin disease.

Reviewing the literature, Kühl (1973) reported that in 45·9% of the patients who continued to work in their original occupation in spite of their dermatosis, the condition of their skin remained satisfactory. Burrows (1972) showed that only 8% of cases of cement dermatitis were clear on review 10–13 years after initial investigation. It is reasonable to believe that an epidemiological investigation would give a more varied picture, mainly in a more favourable direction.

Testing 221 masons and 145 hodmen from the region of Bergen in Norway, Høvding (1970) demonstrated that 9·0% were positive reactors against dichromate but none of the workers in this study group had asked for vocational training or a disability pension. We are not able to say conclusively from the data in the literature in what percentage of patients an allergy against dichromate urges them to change their work, because prospective studies are hard to undertake and the legislation on occupational dermatoses varies from country to country (Breit, 1973; Pirilä *et al.*, 1971). Høvding (1970) re-examined 302 of 366 workers 5 years later, stressing that the possibility of a favourable course should be kept in mind when assessing the prognosis of the incapacity caused by this disease in elderly workers. Follow-up studies of the medical and social condition of the dichromate allergic patient were done by Borelli & Düngemann (1971); Estoppey (1965); Heinze (1972); Kühl (1973); Peter (1968); Pürschel & Fürst (1972); Reichenberger (1972); Saynisch (1958); Türk (1975) and others. Whereas Hunziker & Musso (1969) claimed a 100% improvement after change of work, recent investigations showed that only 29·7–81% regained a satisfactory skin condition (Burrows & Calnan, 1965; Estoppey, 1965; Heinze, 1972; Peter, 1967; Reichenberger, 1972; Türk, 1975). At any rate the number of episodes of sick leave declines considerably after change of work, although Fregert (1975) stated that a change of occupation did not result in better healing than continuing in the same occupation which caused the dermatitis. Türk (1975) reported that 68·8% of his patients did not stay away from work due to their skin problems for one single day after change of work. In addition Pürschel & Fürst (1972) found that in 62% of their cases the new occupation was improperly chosen for preventing relapses. Therefore these results could be improved by paying more attention to this fact. Of course contact with dichromate in every day life plays an important part (Bandmann & Dohn, 1967; Bandmann & Fregert, 1973). Twenty-nine per cent of Türk's patients admitted non-occupational contact (1975). Summing up the influence of change of work, Türk (1975) stated that 78·7% of his 108 patients experienced a distinct or very considerable diminution of the intensity of their skin problems and 51·6% a distinct or very considerable diminution of the frequency of relapses.

Publications about the social situation after change of work caused by an occupational dermatosis showed more or less comparable results, an unsatisfactory social situation after change of work ranging from 15·4 to 70% (Heinze, 1972; Kühl, 1973; Peter, 1967; Pürschel & Fürst 1972; Saynisch, 1958; Türk, 1975).

Türk (1975) confirmed a direct relation between the age of the patient and the social situation. In the age group 19–24 years only 28·6% suffered a deterioration in their economic situation, whereas 28·6% managed to improve it; but in the age group 55–61 years only 16·6% could preserve their social status, whereas all others experienced a sudden drop in their occupational status. This can be explained by the fact that older patients do not learn a new trade but take casual jobs.

The review of the available literature shows that the prognosis of the dichromate allergic patient is poor, taking into account his medical and social outcome. To improve this situation the prevention of sensitization would be the best solution; this would mean the strict avoidance of prolonged contact with dichromate-containing material. If sensitization has occurred the amount of information about the allergen is correlated to the condition of the skin (Türk, 1975). If, on questioning, the patient was not able to name the allergen the skin condition was unsatisfactory in 49% of the cases, but in 76·9% the skin condition was satisfactory if the allergen was known. If the patient was not only aware of the allergen but had been informed exactly about the whole variety of substances which could contain it, the skin condition was satisfactory or even good in 80% of the cases (Türk, 1975). From this the urgency of a special training programme for the allergic patient can be deduced. For this it is essential that the doctor himself has knowledge about suitable jobs for his patients (Pürschel & Fürst, 1972). The problem of the diagnosis of cement dermatitis is solved by incriminating the dichromate ion as the causative allergen; the effect of the dichromate allergy on the patient needs further concentrated attention.

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REFERENCES

BANDMANN, H.-J. & BREIT, R. (1974) Die medikamentöse Kontaktdermatitis. *Internist*, 15, 47.

BANDMANN, H.-J. & DOHN, W. (1967) *Die Epicutantestung*. Bergmann, München.

BANDMANN, H.-J. & FREGERT, S. (1973) *Epicutantestung*. Springer, Berlin.

BORELLI, S. & DÜNGEMANN, H. (1971) Beiträge zur Rehabilitation von chronischen Hautkrankheiten und Allergien. *Schriftenreihe der Bayerischen Landesärztekammer*, 20.

BREIT, R. (1973) Zur Problematik der Rationalisierung der Erfassung von Berufsdermatosen. *Berufsdermatosen*, 21, 95.

BURROWS, D. (1972) Prognosis in industrial dermatitis. *British Journal of Dermatology*, 87, 145.

BURROWS, D. & CALNAN, C.D. (1965) Cement dermatitis, II. Clinical aspects. *Transactions of the St John's Hospital Dermatological Society*, 51, 27.

CRONIN, E. (1971) Chromate dermatitis in men. *British Journal of Dermatology*, **85**, 95.

EBERHARTINGER, CH. & EBNER, D.H. (1968) Beiträge zur Kenntnis der Chromatallergie.2.Mitteilung. *Berufsdermatosen*, **16**, 97.

ESTOPPEY, A. (1965) Etude catamnestique de 105 cas d'eczéma au ciment. *Thèse*, Lausanne.

FREGERT, S. (1975) Occupational dermatitis in a 10-year material. *Contact Dermatitis*, **1**, 96.

FREGERT, S., HJORTH, N., MAGNUSSON, B., BANDMANN, H.-J., CALNAN, C.D., CRONIN, E., MALTEN, K.E., MENEGHINI, C.L., PIRILÄ, V. & WILKINSON, D.S. (1969) Epidemiology of contact dermatitis. *Transactions of the St John's Hospital Dermatological Society*, **55**, 17.

HEINZE, H. (1972) Zum Schicksal beruflich Geschädigter unter besonderer Berücksichtigung Ekzemkranker. *Inaugural Dissertation*. Düsseldorf.

HØVDING, G. (1970) Cement eczema and chromium allergy. An epidermiological investigation. *Thesis*. The University of Bergen, Norway.

HUNZIKER, N. & MUSSO, E. (1960) A propos de l'eczéma au ciment. *Dermatologica (Basel)*, **121**, 204.

KLIGMAN, A.M. (1966) The identification of contact allergens by human assay. III. The maximization test: a procedure for screening and rating contact sensitizers. *Journal of Investigative Dermatology*, **47**, 393.

KÜHL, M. (1971) Über die Bedeutung der Rehabilitation an Berufsdermatosen Erkrankter. *Arbeitsmedizin, Sozialmedizin, Arbeitshygiene*, **6**, 105.

KÜHL, M. (1973) Zur Aufgabe der beruflichen Beschäftigung von Ekzemkranken. Erläuterungen zu diesem Teil der Nr. 46 der Liste der Berufskrankheiten-Verordnung vom 20.6.1968. *Berufsdermatosen*, **21**, 159.

PETER, K. (1968) Über das Schicksal der Patienten mit Gewerbeekzem. *Dermatologica (Basel)*, **136**, 236.

PIRILÄ, V., FREGERT, S., BANDMANN H.-J., CALNAN, C.D., CRONIN, E., HJORTH, N., MAGNUSSON, B., MAIBACH, H.I., MALTEN, K.E., MENEGHINI, C.L. & WILKINSON, D.S. (1971) Legislation on occupational dermatoses. *Acta dermato-venerologica (Stockholm)*, **51**, 141.

PÜRSCHEL, W. & FÜRST, G. (1972) Berufsbedingtes Kontaktekzem, Katamnese und Rehabilitation. *Berufsdermatosen*, **20**, 174.

REICHENBERGER, M. (1972) Katamnestische Untersuchungen bei Angehörigen des Baugewerbes. *Berufsdermatosen*, **20**, 271.

SALFELD, K. (1968) Statistische Auswertung der Berufsdermatosen im Marburger Raum, im Vergleich zu anderen Einzugsgebieten. *Die Berufsdermatosen* 133 (Ed. by G.Weber). Enke, Stuttgart.

SAYNISCH, F. (1958) Zur Wiedereingliederung in den Arbeitsprozess (Rehabilitation) bei beruflichen Hautkrankheiten. *Deutsches Gesundheitswesen*, **13**, 432.

TÜRK, B.M. (1975) Katamnestische Untersuchungen bei Berufsekzematikern unter besonderer Berücksichtigung der Sensibilisierung gegenüber Dichromat-Ionen. *Inaugural Dissertation*. Universität München.

WAGNER, G. & BREDAHL, M. (1965) Untersuchungen zur Frage des beruflichen Hauterkrankungsrisikos. *Berufsdermatosen*, **13**, 1.

British Journal of Dermatology (1976) 94, 353.

News and Notices

Conference on Scabies and Pediculosis

A national conference on scabies and pediculosis will be held on 22–23 May, 1976, at the Medical School, University of Minnesota. The conference was developed under the auspices of the Task Force on Comparative Dermatology for the National Program Council of the American Academy of Dermatology and is supported, in part, by the National Institutes of Health. This symposium will bring together the current scientific and clinical information to physicians and other health care professionals concerned with the identification, treatment and/or control of these conditions.

For further information contact Drs Milton Orkin and Howard I. Maibach or the Office of Continuing Medical Education, Box 293 Mayo Memorial Building, University of Minnesota, Minneapolis 55455.

Postgraduate Course. Techniques for Dermatologic Research in the Office. 8 & 9 October, 1976. University of California, San Francisco

This limited enrollment seminar will stress a pathophysiological approach to just what can be done in 'office' clinical and laboratory research. The staff will stress straight-forward practical solutions to problems in clinical research.

This program is presented by the Department of Dermatology, and Extended Programs in Medical Education, University of California School of Medicine, San Francisco, California.

For information write to Extended Programs in Medical Education, Room 569-U, University of California, San Francisco, California 94143.

Dermatoxicology

There will be a 2-day review in depth of the relevent knowledge of animal and human experimental models in Dermatology presented by the Department of Dermatology and Extended Programs in Medical Education, University of California Medical Center, San Francisco on 3 & 4 February, 1977. This program is orientated for the toxicologist, the internist and dermatologist interested in the determination of hazards from topically applied chemicals and drugs.

For information, please write to Extended Programs in Medical Education, University of California, Room 569 U, 3rd and Parnassus, San Francisco 94143 or call (415) 666-4251.

Appointments

Lancashire Area Health Authority—Preston District

Dr Saikia has been appointed Consultant Dermatologist to the Preston District of Lancashire Area Health Authority. Dr Saikia was educated at Assam, India and graduated M.B., B.S., from the University of Gauhati, Assam, India in 1960. His junior post-graduate work took place in the Assam Medical College Hospital, India, and he was a Registrar in Dermatology there from 1963 to 1965. He then came to this country and obtained the Diploma Certificate in Venereology at Liverpool University in 1966 and eventually a Ph.D., from the University of Glasgow, in 1973. He was Registrar in Dermatology at the Western Infirmary, Glasgow from 1967 to 1973, Senior Registrar in Venereology at the Western Regional Hospital Board from 1973 to 1974 and Senior Registrar in Dermatology at the Western Infirmary, Glasgow from 1974 to 1975. His research interests are the immunological study of psoriasis, and organ culture and its influence on immunological reactions. He has published a number of papers on pemphigus and its association with metastatic disease.

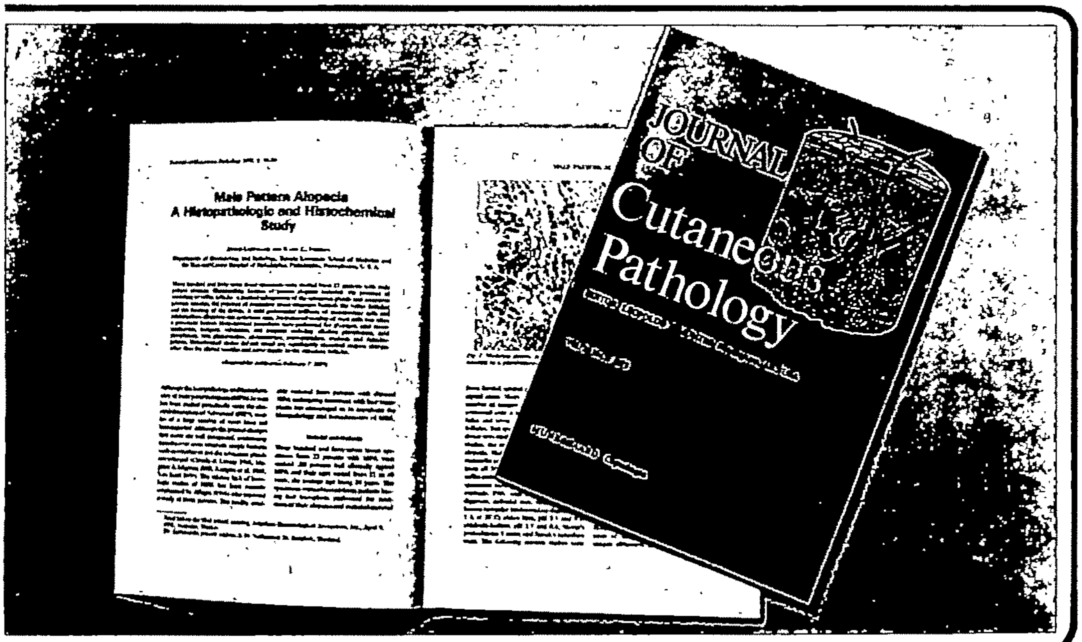
Army Medical Services

At a meeting of the Armed Services Consultant Approval Board of the Royal College of Physicians, London, the following dermatologists were advanced to consultant status.

Colonel D.Sim Davis, RAMC was appointed consultant in respect of his post as dermatologist to the Cambridge Military Hospital, Aldershot. He graduated from the London Hospital in 1950 and held junior dermatology appointments there and at the Radcliffe Infirmary, Oxford. He studied for the Diploma of Dermatology and was joint winner of the Chesterfield Medal in 1971. His published work includes papers on contact dermatitis and histopathology.

Lt Col. A.G.Jarrams, RAMC was appointed consultant in respect of his post as dermatologist to the British Army and its families in Germany at the British Military Hospital, Rinteln. He graduated M.B., B.Chir. from Birmingham in 1948 and obtained the Diploma of Dermatology (London) with distinction in 1973. He has served as a dermatologist in Germany, Singapore and Hong Kong in which latter two posts he made an extensive and recently published survey of leprosy in the Gurkha Brigade of the British Army.

Dermatology



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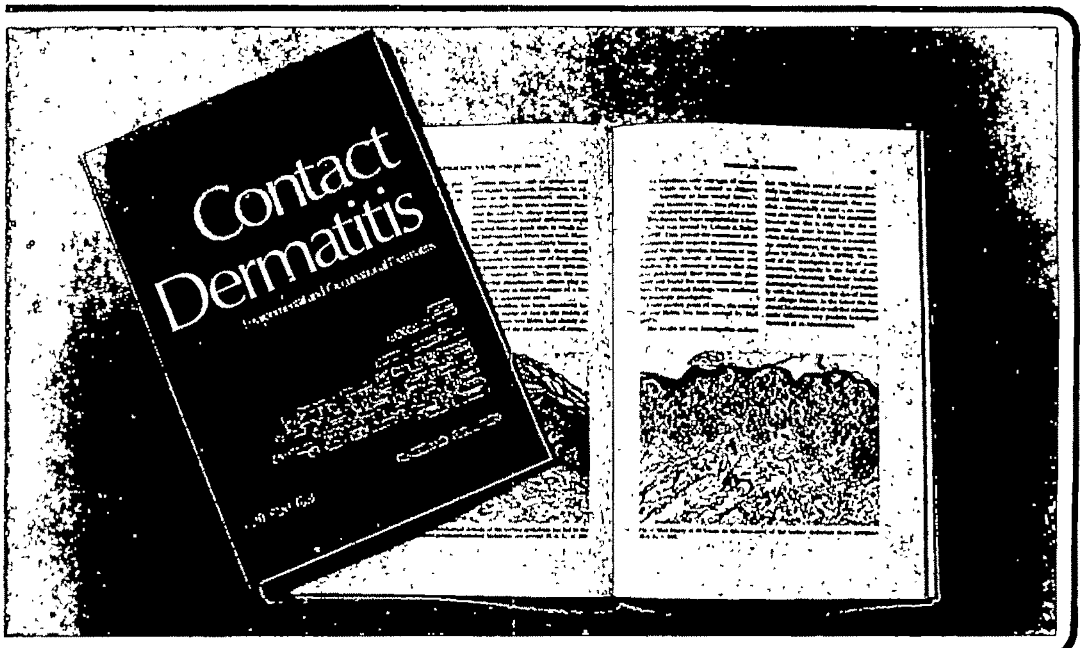

Contact Dermatitis

Environmental and Occupational Dermatitis

Editor: C. D.Calnan, London

Contact Dermatitis is designed primarily as a journal for clinicians who are interested in various aspects of environmental dermatitis. This includes both allergic and irritant (toxic) types of contact dermatitis, occupational (industrial) dermatitis and consumers' dermatitis from such products as cosmetics and toiletries.
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Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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References 1. Whitefield, M. and McKenzie, A. W., Brit. J. Derm. (1975), **92,** 585. 2. Fredriksson, T. and Gip, L., Dermatology Digest. (1976) **Jan.** 27. 3. Barry, B. W. and Woodford, R., Brit J. Derm. Accepted for publication.

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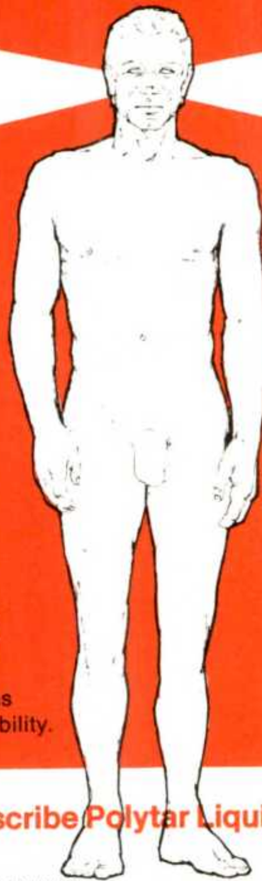

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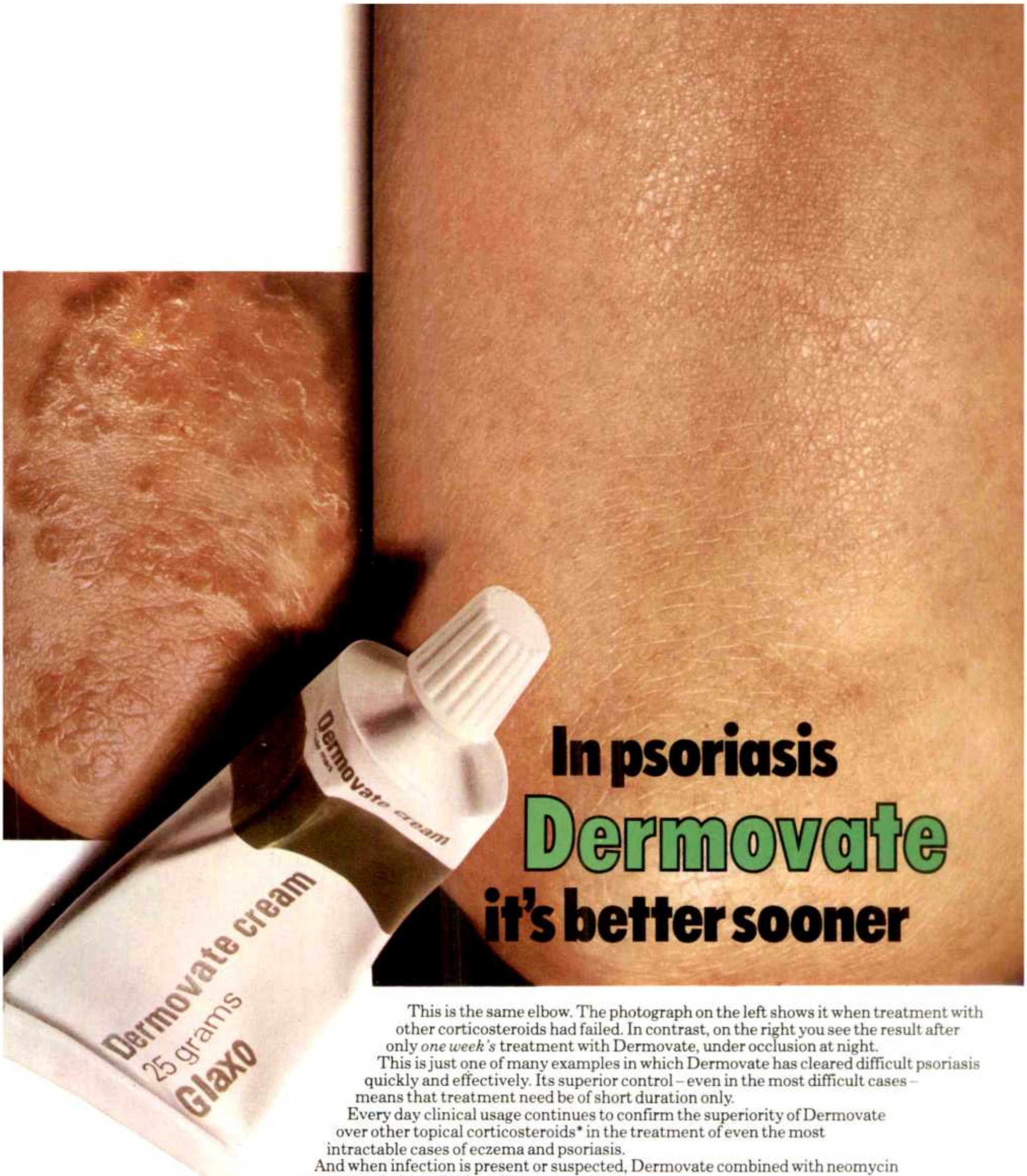

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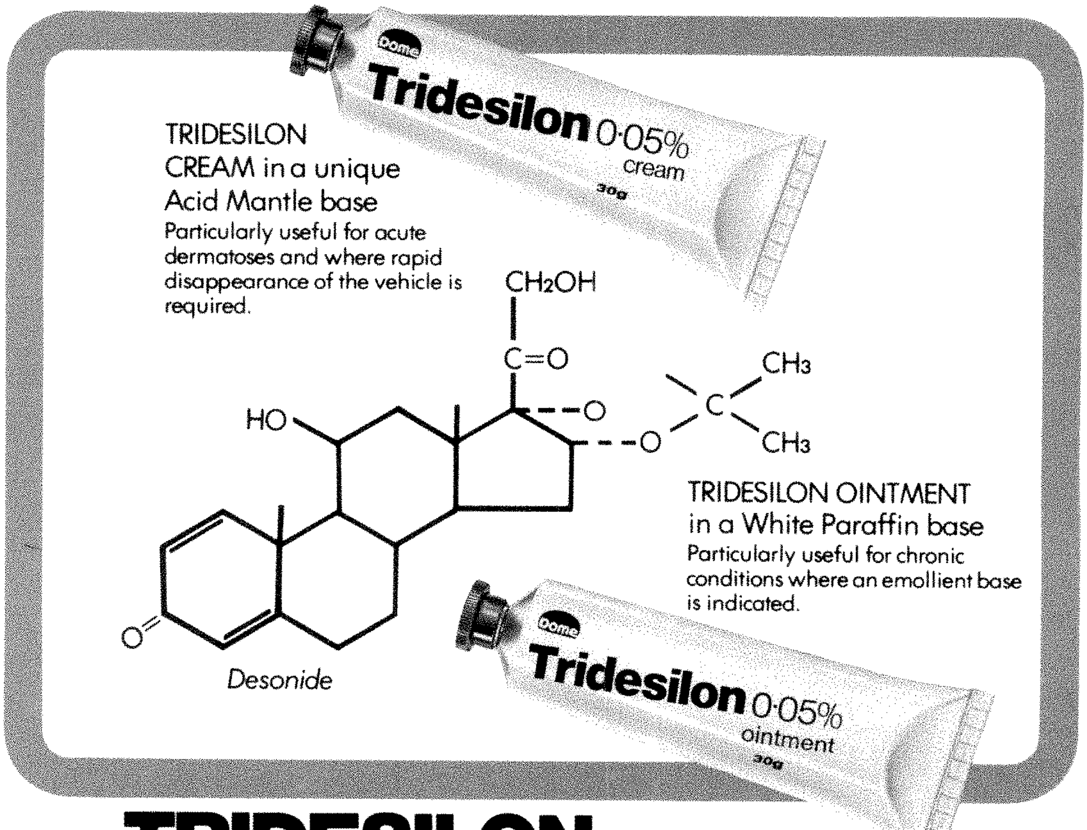

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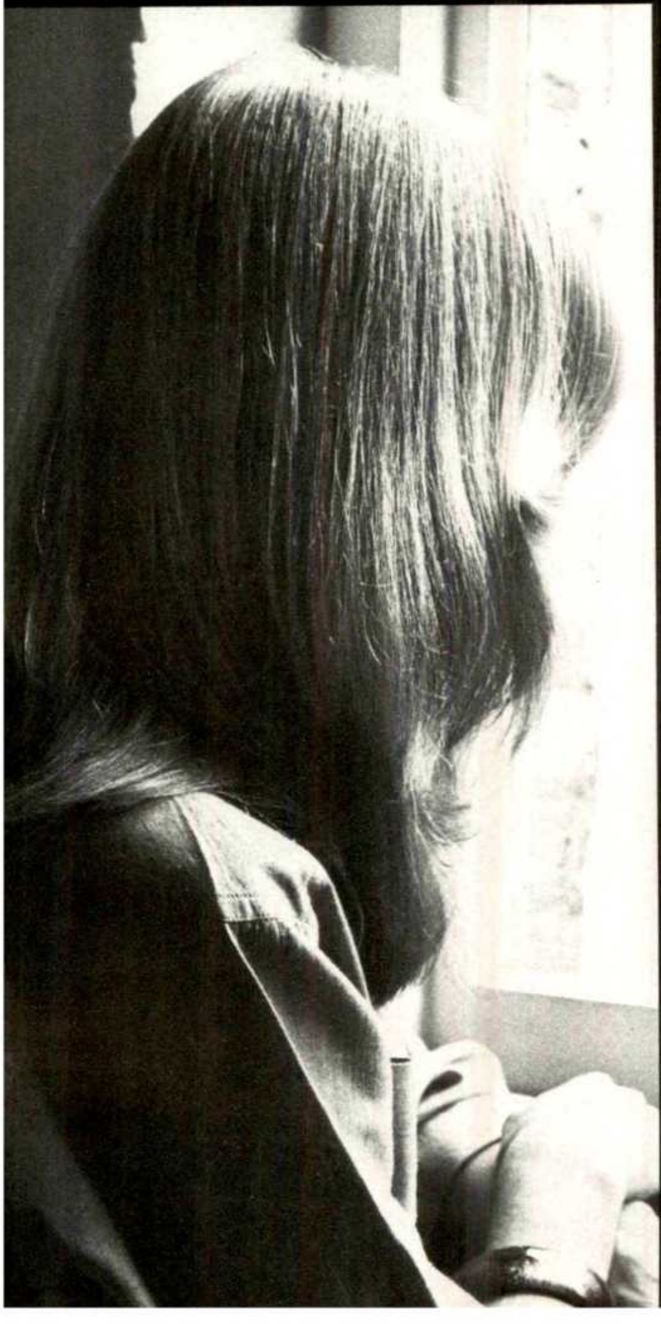

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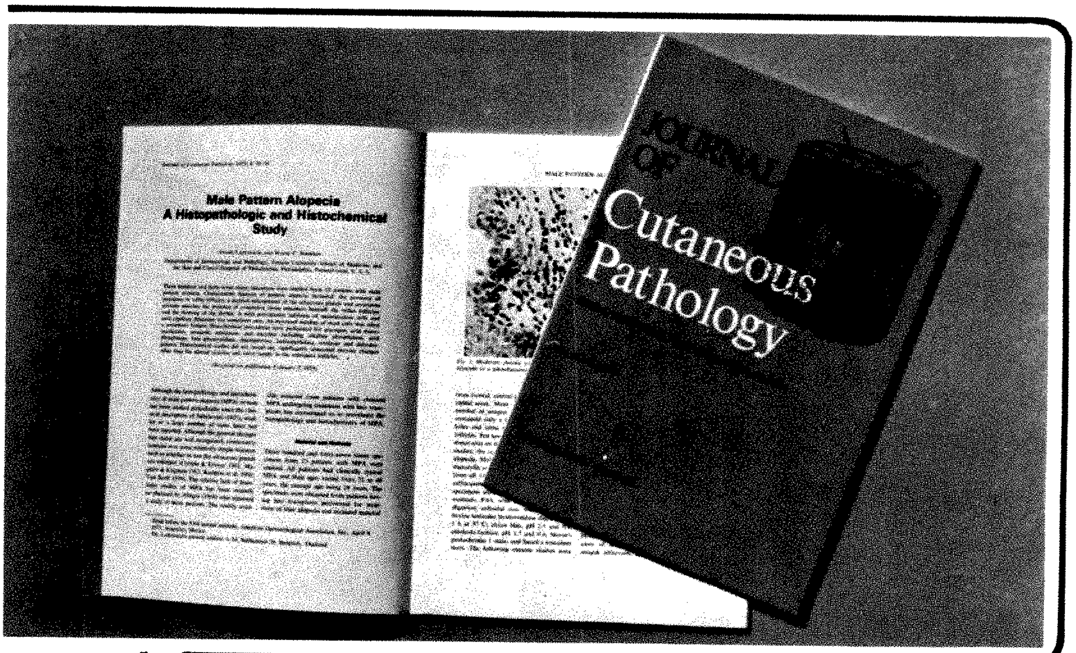

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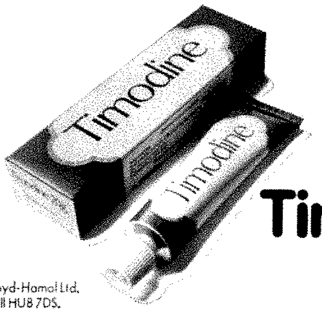

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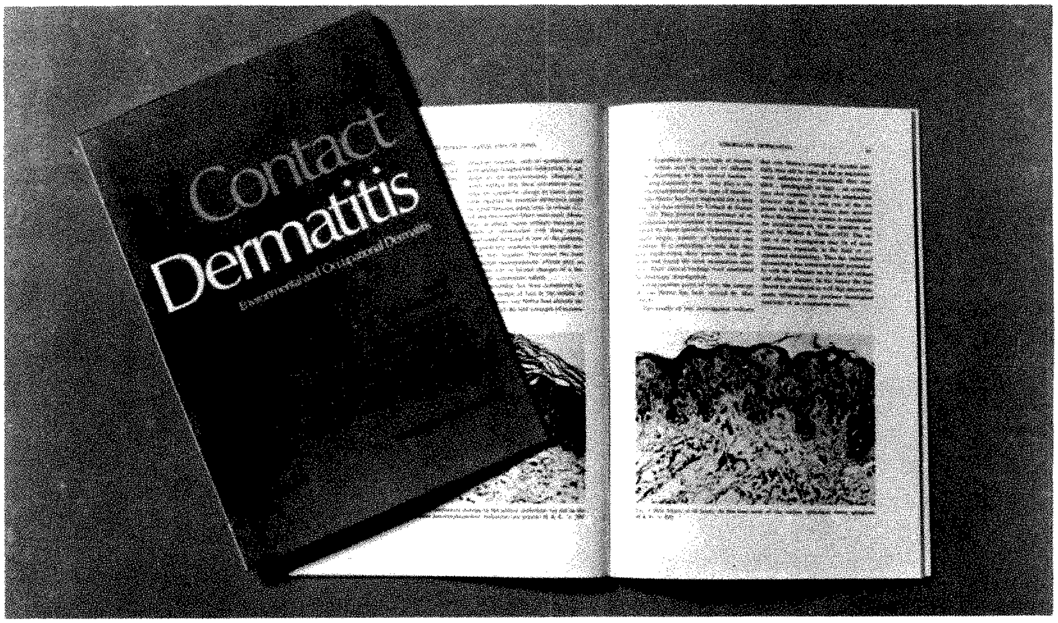

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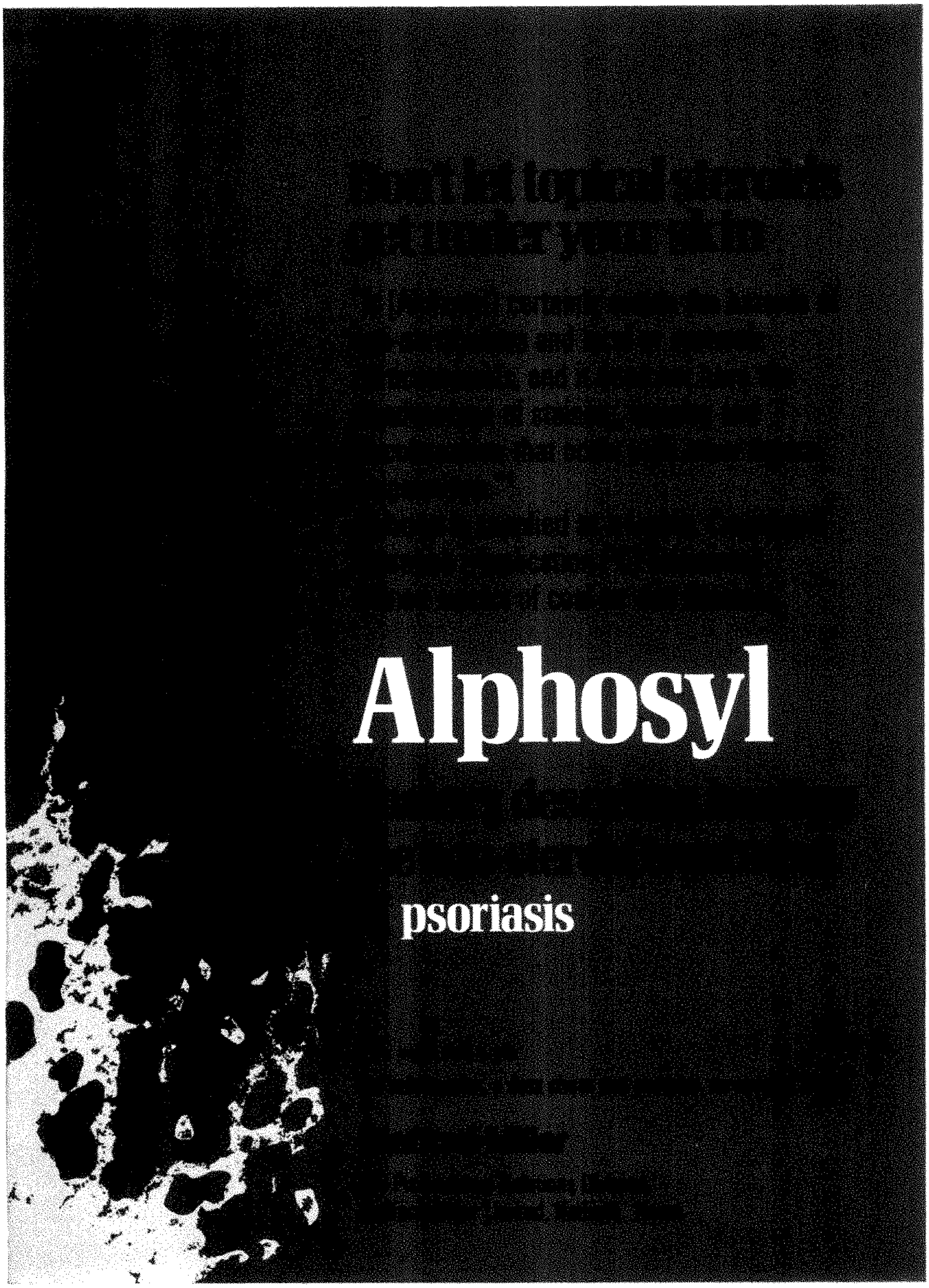

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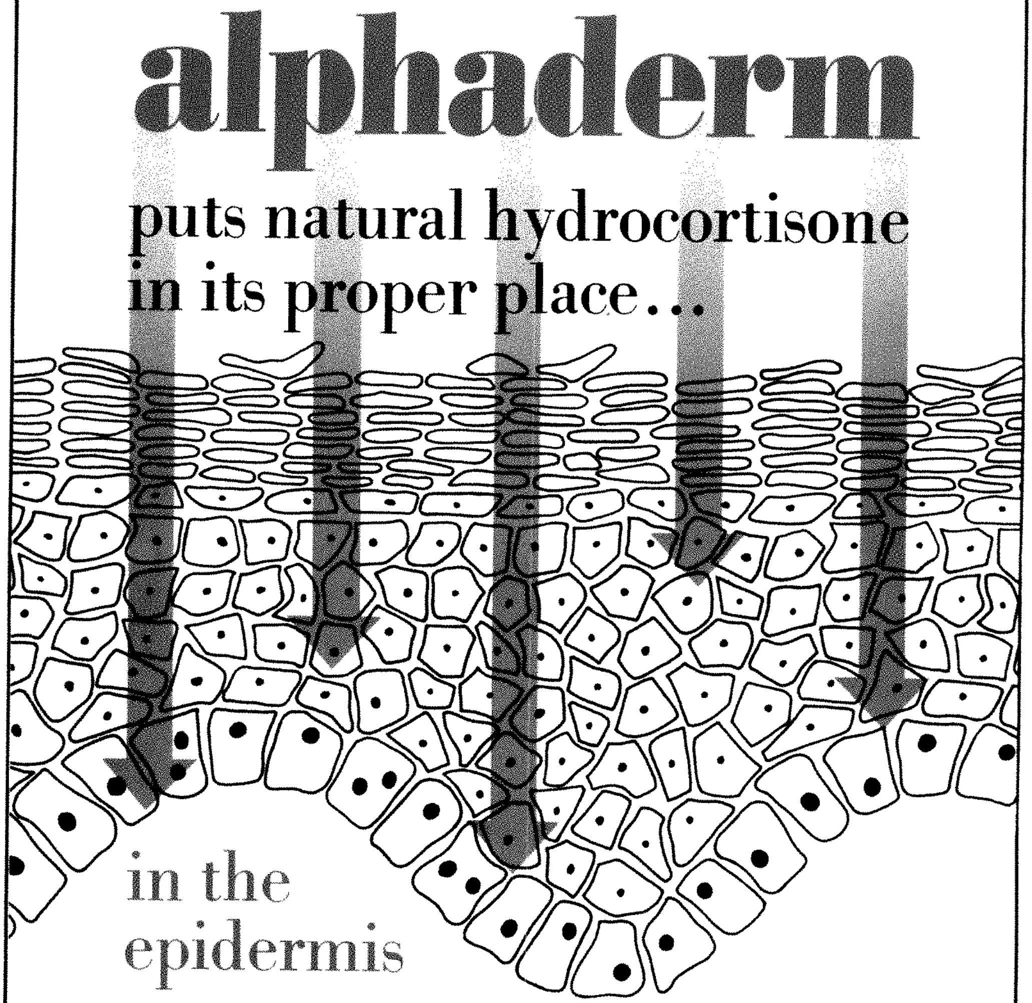
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1. Almeyda, J. and Burt, B.W. (1974) *Br. J. Dermatol.* **91,** 579
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British Journal of Dermatology (1976) 94, 355.

Clinical and Laboratory Investigations

The cell proliferation kinetics of psoriasis examined by three *in vivo* techniques

MARK DUFFILL, NICHOLAS WRIGHT* AND SAM SHUSTER

University Department of Dermatology, and *University Department of Pathology,
Royal Victoria Infirmary, Newcastle-upon-Tyne

Accepted for publication 17 October 1975

SUMMARY

The cell proliferation kinetics of the germinative compartment in psoriasis have been re-investigated with tritiated thymidine and vincristine techniques. The mean cell cycle durations calculated from an extended fraction of labelled mitoses experiment were $T_c = 56$ h, $t_1 = 43$ h, $t_s = 7·7$ h and $t_2 = 5·6$ h. Combination of the results of the FLM experiment with the experimental labelling index and the experimental birth rate suggests a growth fraction of unity and an exponential age distribution for the germinative population. An exponential age distribution is supported by the presence of several layers of germinative cells and the direction of the mitotic axes during cell division.

Psoriasis is characterized by increased production of epidermal cells. The two previous studies of the cell cycle in psoriasis by Weinstein & Frost (1968) and Goodwin, Hamilton & Fry (1974) have given very different estimates of the cell cycle time and these differences have not been resolved (Weinstein *et al.*, 1975). Furthermore, to calculate the cell cycle duration from the experimental data of these authors the age distribution and growth fraction of the germinative population must be known whereas present evidence regarding these parameters is inconclusive. We therefore decided to re-investigate the problem with techniques which enable a direct estimation to be made of the cell cycle durations and also an indirect estimation of the growth fraction and age distribution of the germinative population.

PATIENTS AND METHODS

Twenty-five patients with psoriasis were studied and in each case consent was obtained after an explanation of the nature and purpose of the procedure. Patients with *stable* psoriatic plaques on the lower back were selected for study. No attempt was made to standardize the time of day of the experiments.

In preliminary experiments conducted on ten patients we determined the minimum dose of vincristine sulphate ('Oncovin', Lilly) giving maximal metaphase arrest after intradermal injection into a psoriatic plaque. A 5/8 in. 25 gauge needle was inserted in the uninvolved skin close to the plaque and then advanced until its tip lay under the site in the plaque to be studied. By virtue of a mark made on the hub of the needle to indicate the side of the bevel, the needle was positioned with the bevel facing upwards before the injection was given. From a dose–response curve (to be published), a dose of 1 μg of vincristine in 0·1 ml of normal saline was selected for use in subsequent experiments designed to estimate the rate of entry to mitosis. In these later experiments a single psoriatic plaque was studied in each of eight patients (3 males and 5 females aged 32–73 years). Three separate sites around the edge of the plaque were injected with vincristine and biopsies were then taken at 0·75, 1·5 and 2·5 h after injection. For determination of the native mitotic index a biopsy was also taken from an uninjected site in the same plaque. A 3 mm punch was used with 2% lignocaine as local anaesthetic. The biopsy specimens were fixed in Carnoy's solution, processed by routine methods, cut in 4 μm sections and stained with Harris's Haematoxylin. The slides were viewed under an oil immersion lens and the number of mitoses in 6000 germinative cells was counted for each of the sites. These indices were then plotted against time for each patient.

In further experiments the pulse-labelling index was determined in the psoriatic plaques of six patients (4 males and 2 females aged 43–75 years). In each patient 0·1 ml (10 μCi) of tritiated thymidine (^{3}HTdR) (Specific activity 5·0 Ci/mmol) was injected intradermally and the site was biopsied 1 h later. The specimens were processed, cut at 4 μm and autoradiographs prepared in the standard manner. The autoradiograph sections were examined under an oil immersion lens and the number of labelled cells in 8000 germinative cells was counted for each biopsy. A cell was accepted as labelled if it had at least five grains over the nucleus. The identification of germinative cells is subjective and the criteria which we used for their identification were the same as those described by Weinstein & Frost (1968). These authors also used a correction factor for their labelling index which they determined from 6–7 μm sections. This is to allow for those ^{3}HTdR-incorporating cells which lie too deep in the section for their β emissions to reach the overlying emulsion. Whether or not this is necessary for 6–7 μm sections it appears not to be for 4 μm sections because we obtained peak values of 93% in a fraction of labelled mitoses (FLM) experiment. Moreover, Gelfant (1961) has shown there is no difference between the labelling indexes determined from 2 μm and 7 μm sections.

The technique used for the FLM experiment was the same as that described by Weinstein & Frost (1968) except that biopsies were taken over a longer period after the initial labelling. In a single male patient aged 72 years, twelve separate sites around a psoriatic plaque were labelled at 0 h with 0·1 ml (10 μCi) of ^{3}HTdR and the injected sites were then serially biopsied at predetermined intervals up to 45 h. Autoradiographs were prepared from the biopsy specimens and all labelled mitoses from late prophase to early telophase were counted amongst 100 mitoses for each biopsy. The data thus obtained were analysed by the Gilbert program (Gilbert, 1972) to obtain the values of the best-fitting parameters of the cell cycle (the Gilbert program regards mitosis as instantaneous and this is shared between the pre- and post-synthetic intervals).

RESULTS

The results of the vincristine experiments are shown in Fig. 1 and Table 1. There is no significant difference between the native mitotic index (at 0 h) and the metaphase index at 0·75 h; the rate of metaphase accumulation has therefore been calculated between 0·75 and 2·5 h after vincristine injection. The regression coefficient of this line gives the rate of metaphase accumulation over this time interval, and at steady-state conditions, this is a measure of the birth rate. The mean birth rate was 12·72 ± 1·72 cells/1000 germinative cells/h.

The pulse-labelling indices for the six patients are shown in Table 2. The mean index was 103 cells ±22 cells/1000 germinative cells. Fig. 2 shows the percentage of labelled mitoses at intervals up to

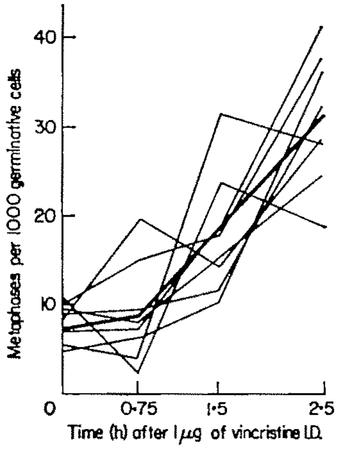
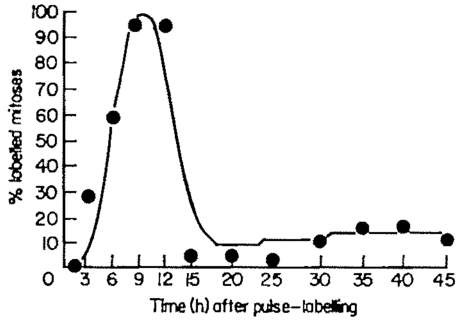


FIGURE 1. Metaphase index in psoriatic plaques as a function of time after intradermal injection of 1 μg vincristine. The lightly drawn lines are the values for individual plaques and the heavy line is the overall mean. The calculated mean rate of entry to mitosis is 12·7 cells/1000 cells/h.

TABLE 1. Metaphase index in psoriatic plaques following intradermal injection of 1 μg of vincristine (8 patients)

Time after injection (h)	Metaphases per 1000 germinative cells ± s.e.
0	7·94±0·95
0·75	9·36±2·02
1·5	18·47±2·49
2·5	31·59±2·70



Time (h)	1	3	6	9	12	15	20	25	30	35	40	45
PLM	0	28	59	93	93	4	4	2	10	16	16	11

FIGURE 2. The percentage of labelled mitoses in a psoriatic plaque as a function of time after pulse labelling. The curve is computer fitted to the data.

TABLE 2. Labelling index in psoriatic plaques pulse—labelled with 10 μ Ci of ^{3}HTdR

Patient no.	Labelled cells per 1000 germinative cells
1	52
2	107
3	202
4	63
5	117
6	78
Mean ± s.e.	103 ± 22

TABLE 3. Mean cell cycle durations in psoriatic plaque calculated by FLM method

	T_c	t_{g1}	t_s	t_{g2}
Mean	56·45	43·16	7·74	5·56
s.e.	2·92	2·93	0·58	0·28
	CV_c		$CV_s \pm {}_{g2}$	CV_{g2}
Mean	74·99		13·13	32·42
s.e.	12·03		1·23	52·85

CV = coefficient of variation.

45 h after pulse-labelling and a curve fitted to the experimental data by the Gilbert program. Table 3 shows the mean cell cycle time and phase durations calculated by the program and their standard errors and coefficients of variation. The mean durations were: $T_c = 56$ h, $t_1 = 43$ h, $t_s = 7{\cdot}7$ h and $t_2 = 5{\cdot}6$ h (where T_c is the cell cycle time, $t_1 = \frac{1}{2}t_m$ (the duration of mitosis) $+ t_{g1}$ (the pre-synthetic interval) and $t_2 = \frac{1}{2}t_m + t_{g2}$ (the time interval between DNA synthesis and mitosis)).

Calculation of the cell kinetic parameters of the germinative compartment from the FLM durations, the pulse-labelling index and the stathmokinetic birth rate

In the analysis of our results we have used the formulae listed in Table 4. The 'growth fraction' is the proportion of the germinative population that is actively progressing around the cell cycle. The

TABLE 4. Cell cycle formulae for rectangular and exponential age distribution in a germinative population (partly after Cleaver, 1967)

$$I_s\ (\text{theoretical}) = \left(\exp\frac{t_s(\text{FLM})\ln 2}{T_c(\text{FLM})} - 1\right)\left(\exp\frac{t_2(\text{FLM})\ln 2}{T_c(\text{FLM})}\right) \tag{1}$$

(exponential)

$$I_s\ (\text{theoretical}) = \frac{t_s(\text{FLM})}{T_c(\text{FLM})}\ (\text{rectangular}) \tag{2}$$

$$I_p = \frac{I_s\ (\text{experimental})}{I_s\ (\text{theoretical})}\ (\text{rectangular or exponential}) \tag{3}$$

$$T_c = \frac{\ln(1+I_p)}{K_b(\text{vc})}\ (\text{exponential and growth factor}) \tag{4}$$

$$T_c = \frac{I_p}{K_b(\text{vc})}\ (\text{rectangular and growth fraction}) \tag{5}$$

I_s = proportion of germinative cells synthesizing DNA
t_s = duration of DNA synthesis
T_c = cell cycle time
t_2 = $t_{g2} + \frac{1}{2}t_m$

ln = natural logarithm
exp = exponential function
vc = vincristine data
FLM = fraction of labelled mitosis curve data
I_p = growth fraction

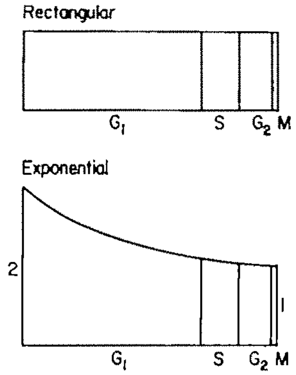


FIGURE 3. Two types of age distribution of a germinative cell population in steady-state conditions. The 'rectangular' age distribution has cells evenly distributed round the cell cycle, the 'exponential' age distribution refers to a population in which numbers fall off exponentially with cycle age and the ratio of new daughter cells to mitoses is 2:1.

term 'rectangular age distribution' refers to a germinative population in which the cells are evenly distributed around the cell cycle, and this age distribution is associated with steady-state conditions.

The term 'exponential age distribution' refers to a population in which cell numbers fall off exponentially with cycle age and the ratio of new daughter cells to mitoses is 2:1. In the stable psoriatic plaques studied, this age distribution is assumed to be associated with *overall* steady-state conditions. The two age distributions are illustrated in Fig. 3.

The FLM data can be taken first because this method estimates cell cycle durations independently of age distribution and growth fraction. These durations are referred to as 'theoretical' and those obtained from the vincristine data as 'experimental'. If $T_c = 56$ h, $t_1 = 43$ h, $t_s = 7{\cdot}7$ h and $t_2 = 5{\cdot}6$ h:

The theoretical labelling index (LI) for an exponential age distribution

$$= 0{\cdot}10 \tag{1}$$

The theoretical labelling index for a rectangular age distribution

$$= 0{\cdot}14 \tag{2}$$

Next, using our experimental LI of 0·10,

For experimental LI of 0·10, theoretical LI of 0·10 and exponential age distribution

$$\text{the growth fraction} = 1{\cdot}0 \tag{3}$$

For experimental LI of 0·10, theoretical LI of 0·14 and rectangular age distribution

$$\text{the growth fraction} = 0{\cdot}71 \tag{3}$$

Finally, introducing our experimental birth rate of 0·01272 cells/cell/h:

For exponential age distribution and growth fraction of unity

$$T_c = 54{\cdot}5 \text{ h} \tag{4}$$

For rectangular age distribution and growth fraction of 0·70

$$T_c = 55{\cdot}8 \text{ h} \tag{5}$$

The cell cycle times obtained from the stathmokinetic date agree closely with the FLM time under either of the two sets of conditions for the germinative population.

DISCUSSION

The delay in onset of action of vincristine which we observed has also been noted by other workers. It has been suggested that cells in mitosis at the time of injection of the vincristine complete division, and that the delay period is therefore a measure of the duration of mitosis. Although there was no significant difference between the metaphase index at 0 and 0·75 h, inspection of the metaphase accumulation graph suggests that the delay period may be somewhat shorter than we have assumed and that the duration of mitosis in the psoriatic plaque is therefore less than 0·75 h. In support of this, the mitotic durations calculated from the mitotic index and birth rate and from the mitotic index and FLM-derived cell cycle time were 0·62 and 0·64 h respectively. These figures may be compared with the mitotic durations of 0·3 h and 0·5 h calculated by Weinstein & Frost and by Goodwin *et al.* respectively for psoriasis, and with the mitotic duration of 1 h reported by the former authors for normal epidermis.

For either of the combinations of growth fraction and age distribution that we have proposed for the germinative population in psoriasis the rate of entry to mitosis will be the same. However, there are limitations to the use of the vincristine method to measure the true rate in a steady-state exponentially distributed germinative population. This is because in such a population migration of germinative cells to the differentiating compartment is thought to be dependent upon the 'population pressure' created by cell birth. Thus when cell birth is blocked by vincristine, migration will cease, and the rate of entry to mitosis will rise exponentially during the period of vincristine action. However, during the first 1·75 h of vincristine action in a germinative population where the cell cycle time is 56 h, the rate of entry to mitosis is near-linear and does not differ significantly from the rate in the undisturbed plaque. Measurement of the true rate of entry to mitosis can therefore be made during this early phase.

A possible source of error in determination of the labelling and mitotic indices in the individual patient is the variation which may be present with time in the proportion of germinative cells occupying the S and M phases. The rate of entry of cells to a phase may vary due to synchronization between individual cell cycles or changes in the growth fraction and there may be a circadian rhythm in the durations of DNA synthesis and mitosis. The evidence for such variation in psoriasis is conflicting: Milstein & Cornell (1973) found no variation in the mitotic index in psoriatic plaques over a 24-h period, whereas *in vitro* labelling studies by Hell & Hodgson (1966) and Pullman, Lemartz & Steigleder (1974) showed periodic variation in the labelling index. When the labelling index and mitotic index vary with time, it is necessary to determine average values for use in calculations of the cell cycle time or growth fraction.

Our FLM durations are based upon the study of a single patient and, partly because of the limitation placed upon the number of biopsies, wide coefficients of variation are apparent. Nevertheless, by spacing out the biopsies so as to extend the period of experimental observation, a potentially more accurate T_c value is obtained than by the previous authors who have used biopsies which are more closely spaced in time. Their method gives a more accurate measure of t_s but the curve has then to be extrapolated to obtain T_c. The possibility of missing a second peak seems unlikely and the hourly biopsies required would be unacceptable.

Although our cell cycle durations can be regarded as only provisional, it is of interest that similar values have been obtained with the FLM method for psoriatic cells in tissue culture (Chopra & Flaxman, 1974). These authors report: $T_c = 53{\cdot}5$ h, $t_{g1} = 38{\cdot}9$ h, $t_s = 6{\cdot}5$ h and $t_{g2} = 7$ h.

In order to calculate the growth fraction from the experimentally determined proportion of cells in DNA synthesis and the cell cycle durations, the age distribution of the germinative population must be known. The growth fraction can then be calculated from the difference between the observed and predicted proportion of cells in the S phase. If a rectangular age distribution is assumed the calculated growth fraction is 0·71, and if an exponential age distribution is assumed, the calculated growth fraction is unity. It seems likely that in the rapidly dividing psoriatic skin the growth fraction would be unity and thus would favour an exponential age distribution. A consideration of the structure of the germinative population and of the movement of the dividing cells in the plaque also favours an exponential age distribution.

Because only stable psoriatic plaques have been studied, steady-state conditions may be assumed for the proliferative population. On average one daughter cell of each division must enter the differentiating compartment before the other daughter cell divides again.

A rectangular age distribution of the germinative cells will be created if one of each pair of newborn cells immediately joins the differentiating compartment and no migration of older cells occurs. This age distribution can only arise if (i) there is a single layer of germinative cells and (ii) the mitotic axes of the dividing cells are vertical so that the separation of the daughter cells of a division results in the transfer of one cell out of the layer and into the differentiating compartment. By contrast an exponential age distribution will be present if all newborn cells are (i) initially retained in the germinative compartment and (ii) are then subject to a random removal process which (iii) operates throughout the cell cycle.

In a single-layered germinative compartment, retention of both daughter cells will occur if the direction of the mitotic axis is horizontal. In a multi-layered compartment, however, the direction of the mitotic axis will not be the sole factor causing initial daughter cell retention. Only in the superficial layer of such a compartment can mitosis effect the transfer of a daughter cell out of the compartment since both daughter cells of mitoses occurring in deeper layers must at first be retained. We have determined the directions of the mitotic axis in the germinative compartment from 100 anaphase and telophase figures and have found these directions to be: horizontal 58%, oblique 18% and vertical 24%. Thus the directions of the mitotic axis and the multi-layered germinative compartment both support the age distribution we have proposed for psoriasis.

The basis for the random removal of cells from the germinative compartment is thought to be the 'population pressure' created in the compartment when two new cells replace a single dividing cell. It is believed that under this 'pressure' a cell migrates out of the compartment at random, thus maintaining steady-state conditions.

There is no direct evidence as to whether cell removal operates throughout the cycle. From their study of the dorsal epidermis of the hairless mouse, Iversen, Bjerknes & Devik (1968) have proposed that germinative cells migrate only in late G_1, and they quote the work of Marques-Pereira & LeBlond (1965) as evidence that basal cells are firmly bound to the basement membrane after the onset of DNA synthesis. However, in the multi-layered germinative compartment of psoriasis, germinative cells above the basal layer are unlikely to have an attachment to the basement membrane and migration from all phases of the cycle should be possible. Furthermore, migration of S and G_2 cells from the basal layer of mouse skin has been observed when cell production is stimulated (Rohrbach, Hecker & Sandritter, 1968). It seems not unlikely, therefore, that similar migration occurs from the basal layer of psoriatic skin.

Weinstein & Frost (1968) and Goodwin *et al.* (1974) respectively calculated the cell cycle time in psoriasis as 91 h and 37·5 h from the ratio of the experimental duration of DNA synthesis to the experimental labelling index. In these calculations both groups of authors assume a growth fraction of unity and rectangular age distribution for the germinative population. The difference in their calculated times is largely due to the different values they obtained for the experimental labelling index and this difference in turn arises from different interpretations of the extent of the germinative population and from the use by the former authors of a correction factor for their labelling index. (Our own labelling index agrees with that of Goodwin *et al.* and is similarly based upon the count of a large number (8000) of germinative cells and without the use of a correction factor.)

In agreement with both groups of previous authors the present study favours a value of unity for the growth fraction. However, if the age distribution is exponential as our evidence suggests and not rectangular as they have tacitly assumed, the method they use to calculate the cell cycle durations is not valid. This is because in a germinative population with an exponential age distribution the proportion of cells in the S phase is dependent upon the position of this phase in the cell cycle.

The advantage of the extended FLM method which we have used is that the growth fraction, age distribution and variation present with time in the proportion of cells in the cycle phases, do not need to be known in order to calculate the cell cycle durations. Although this method has practical limitations in the number of biopsies and long period of experimental observation it entails, it may be possible to circumvent these difficulties if a composite FLM curve is constructed from a small number of biopsies in a number of individual patients.

ACKNOWLEDGMENTS

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REFERENCES

Chopra, F. & Flaxman, R. (1974) Comparative proliferative kinetics of cells from normal human epidermis and benign epidermal hyperplasia (psoriasis) *in vitro*. *Cell and Tissue Kinetics*, **7**, 69.

Cleaver, J. (1967) Thymidine metabolism and cell kinetics. In: *North-Holland Research Monographs, Frontiers of Biology*, Vol. 6, p. 123, Table 4, I. North-Holland Publishing Co., Amsterdam.

Gelfant, S. (1961) Initiation of mitosis in relation to the cell division cycle. *Experimental Cell Research*, **26**, 395.

Gilbert, G.W. (1972) The labelled mitoses curve and the estimation of the parameters of the cell cycle. *Cell and Tissue Kinetics*, **5**, 53.

Goodwin, P., Hamilton, S. & Fry, L. (1974) The cell cycle in psoriasis. *British Journal of Dermatology*, **90**, 517.

Hell, E. & Hodgson, C. (1966) The uptake of ^{3}H-thymidine by epidermal cells in normal and psoriatic subjects. *British Journal of Dermatology*, **78**, 262.

Iversen, Olf., Bjerknes, R. & Devik, F. (1968) Kinetics of cell renewal, cell migration and cell loss in the hairless mouse dorsal epidermis. *Cell and Tissue Kinetics*, **1**, 365.

Marques-Pereira, J.P. & LeBlond, C.P. (1965) Mitosis and differentiation in the stratified squamous epithelium of the rat oesophagus. *American Journal of Anatomy*, **117**, 73.

Milstein, H. & Cornell, R. (1973) Diurnal mitotic studies of psoriatic epidermis. *Journal of Investigative Dermatology*, **61**, 180.

Pullman, H., Lemartz, K. & Steigleder, G. (1974) *In vitro* examination of cell proliferation in normal and psoriatic epidermis, with special regard to diurnal variations. *Archiv für Dermatologische Forschung*, **250**, 177.

Rohrbach, R., Hecker, E. & Sandritter, W. (1968) Cytophotometrische Messungen des DNS—Gehaltes der Epidermis nach unspezifischen, Cocarcinogen und Carcinogen. *Zeitschrift für Krebsforschung und klinische Onkologie*, **70**, 211.

Weinstein, G. & Frost, P. (1968) Abnormal cell proliferation in psoriasis. *Journal of Investigative Dermatology*, **50**, 254.

Weinstein, G., Goodwin, P., Hamilton, S. & Fry, L. (1975) On the cell cycle in psoriasis (correspondence). *British Journal of Dermatology*, **92**, 229.

British Journal of Dermatology (1976) **94**, 363

Simplification of local therapy of psoriasis with 8-methoxypsoralen

METHODS AND INITIAL RESULTS

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SUMMARY

Local 8-methoxypsoralen black-light treatment was carried out on sixty-two patients. For the first time, oil was employed as a preparation base in order to increase the transparency and so the effectiveness of irradiation. Also the need for a descaling pretreatment was avoided. In investigations still being carried out, 27·5% patients showed very good improvement, 48·4% good and 24·2% unsatisfactory results of treatment. In many cases, less than fifteen irradiation sessions were necessary for clinical healing. However, to obtain a uniform pigmentation, additional irradiation proved necessary.

In recent years the photodynamic effect, particularly of 8-methoxypsoralen (8-MOP) and black-light, has been employed in the therapy of psoriasis (Juhlin, 1974; Mortazawi, 1972; Mortazawi & Oberste-Lehn, 1973; Parrish *et al.*, 1974; Tronnier & Schüle, 1973; Tronnier & Loehning, 1975; Walter & Voorhees, 1973; Weber, 1974a, b, c; Willis & Harris, 1973; Wolff, 1975; Wolff *et al.*, 1975).

TABLE 1. Comparison between external and internal 8-MOP therapy

	External	Internal
Application	Laborious	Simple
Effect	Good	Not always sufficient
Duration of irradiation	Shorter	Longer
Cosmetic effect	Patchy pigmentation	Uniform pigmentation
General side effects	None (?)	Nausea, giddiness, etc. occasional transaminase increases
Side effect on the skin	Limited to area of treatment	Generalized

8-MOP can be used both locally and orally, each having its advantages and disadvantages (Table 1). Since the discussions of the possible systemic side effects of 8-MOP (Becker, 1960; Griffin, 1959; Hopkins *et al.*, 1963; Joseph *et al.*, 1974; Langner *et al.*, 1975; O'Neal & Griffin, 1957; Townsend, Wright & Hopwood, 1971; Urbach, 1959; Urbach, Forbes & Davies, 1975) are yet to be concluded, one of our first aims was to simplify the local application of 8-MOP.

METHODS

Administration

We have employed 8-MOP in an oily preparation for a period of 2 months. The patients rubbed this oil on the trunk and extremities with cupped hands wearing plastic gloves. Prior removal of scales was not necessary. Assistance was only occasionally required for areas on the back. After 60 min, the recumbent patient was irradiated with black-light. Initially each side was irradiated for 5 min, then, with stepwise increases of 2 min, irradiation was applied for up to 15 min per side. The irradiation was undertaken 2–4 times per week at the beginning, then later, 1–2 times per week. In the event of marked erythema or blister formation, the sensitive area was covered or the irradiation treatment interrupted.

8-*MOP oil*

A week's requirement was prepared as 0·15%(w/v) using 8-MOP powder (kindly provided by Basotherm, Biberach) and oleum arachidis (peanut oil) (German Pharmacopoeia 7). The powder dissolved in the oil extremely slowly so that mixing was carried out over 24 h with the aid of a magnet stirrer at room temperature. The thin layer chromatographic control after 1 week showed that no significant decomposition of 8-MOP occurred.

Black-light apparatus

Valves F 20 T 12-BLB from the firms Sylvania or Philips were used. The emission maximum has a wavelength of 355 nm with a 50% energy output in a range of 35 nm (335–370 nm). The energy output was 7·5 W for 315–380 nm. There were a total of twenty-four valves in six cylindrical segments; an irradiation box movable at all three levels (Jupiterlicht Ltd, Berlin, special construction); three irradiation boxes to each side of the treatment couch. The skin was kept 30 ± 20 cm from the light source depending on the degree of infiltration and compatibility.

RESULTS

The evaluation of these preliminary results was based on the following criteria:

Group I. 100% healed (no erythema, no scale formation, no infiltration) but persistent irregular pigmentation.

Group Ia. Area of skin affected is 100% healed; however, during treatment fresh areas become involved.

Group II. 75% of affected area healed; remaining area exhibits reduced involvement.

Group III. 50% of affected area healed, remaining area exhibits reduced involvement.

Group IV. Less than half the affected area healed.

Group V. Unaltered.

Group VI. Deteriorated.

Of the sixty-two patients, twenty-five were men and thirty-seven women; the results were related to the different groups with respect to number of patients as well as to percentage values (Table 2).

TABLE 2. Relationship between the single groups (as defined under 'Results') and the numbers of patients belonging to these groups

Group I–VI	Men n = 25 (100%)	Women n = 37 (100%)	Total n = 62 (100%)
I	4 (16)	8 (21·6)	12 (19·4)
Ia	3 (12)	2 (5·4)	5 (8·1)
II	6 (24)	9 (24·3)	15 (24·2)
III	8 (32)	7 (18·9)	15 (24·2)
IV	0 (0)	5 (13·5)	5 (8·1)
V	3 (12)	4 (10·8)	7 (11·3)
VI	1 (4)	2 (5·4)	3 (4·8)

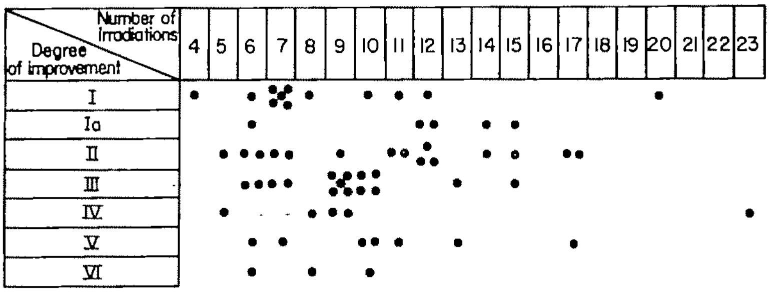

FIGURE 1. The dependence of healing rate on the number of irradiation sessions. Males, n = 25; females, n = 37. Each point represents a patient.

In Fig. 1, the rate of healing is related to the number of times irradiation was undertaken.

The following conclusions can be drawn from Table 2 and Fig. 1:

1. In approximately 20% of cases complete healing was obtained after 6–12 irradiation sessions. In one case irradiation had to be carried out 20 times (Group I).

2. In about 8% of cases, complete healing was seen after 6–15 irradiation sessions. However, fresh areas of up to 1 cm diameter developed during the course of treatment (Group Ia).

3. In about 25% of cases, 75% healing was observed after (till now) 5–17 irradiation sessions (Group II); with 2/3 of the patients this was achieved after 12 or less irradiation sessions.

4. In 25% of cases, 50% healing was observed after (till now) 6–15 irradiation sessions (Group III). Three-quarters of these patients achieved this status after 10 or less irradiation sessions.

5. 25% of cases must be classified at the moment into groups IV, V and VI, after (till now) 5–23 irradiation sessions.

Groups I and Ia with smaller numbers of irradiation sessions consist of patients in a less acute state or a minor extension of the lesion respectively. In one case corticosteroid treatment had already been undertaken. The unsatisfactory results (Groups IV–VI) can be explained in terms of the presence of inveterate lesions or too weak irradiation frequency. Both factors could also concurrently play a role.

Side effects

For a flawless pigmentation further irradiation is necessary after complete healing. The application of oil was found acceptable to the patient.

In 35% of cases, erythema occurred in the intertriginous region and developed into blisters in four cases. After the 5th session of irradiation these complications did develop in one case. Necrosis, however, was never observed. As can be seen from Fig. 2, no correlation between the degree of erythema formation and degree of therapeutic success was found. About half the patients complained of tiredness and increased itching during the irradiation treatment.

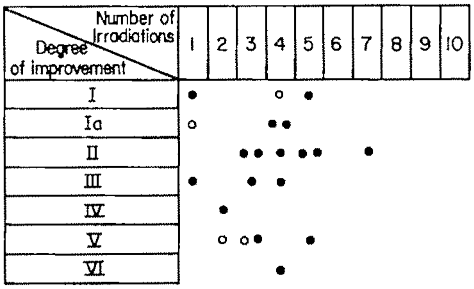

FIGURE 2. The dependence of erythema and blister formation on the number of times irradiated and the healing rate. n = 22, 11 females, 11 males. ● Erythema; ○ erythema and blisters.

DISCUSSION

Our type of treatment combines practically all the advantages of external therapy with those of internal therapy as described in Table 1. A uniform pigmentation was achieved by rubbing the oil preparation onto the whole body with the exception of face and hands. This was also feasible using an alcoholic solution but the cooling effect due to evaporation as well as the degreasing properties were found unpleasant by the patients. The uncovered parts of the body could also be treated to obtain a uniform pigmentation, if exposure to strong sunlight was precluded.

A further advantage over the two procedures previously practised (oral administration and local application in alcohol solution) is that a keratolytic pretreatment is unnecessary. The oil causes a reduction of reflection and scatter of incident light and also makes the scales invisible. The glass-platelets method of measuring skin surface fat according to Schaefer & Kuhn-Bussius (1970) showed that oil increases the skin transparency by 80%, whereas the alcoholic solution causes a 20% decrease due to its evaporation and subsequent 8-MOP crystal formation on the skin. The minimal absorption by the oil of light at 360 nm is insignificant considering the 80% increase of transparency. A further reason for the keratolytic treatment, the decrease of permeation effected by the scales, is not relevant for 8-MOP treatment (Kammerau *et al.*, 1975).

A comparison of our results with those of other workers is difficult because the irradiation appliances employed vary considerably. That our results are somewhat inferior to those obtained by other authors is probably because we treated the patients in the first series only twice weekly for 2–3 weeks and afterwards once weekly.

On the strength of our practical experience we have altered our procedure. Application of 8-MOP oil is combined with daily irradiation until healing is achieved. This corresponds to the practical procedure of most authors and, indeed, takes account of the experience of the patients that the psoriasis improved during the first 2 days after irradiation, but deteriorated thereafter.

REFERENCES

Basotherm GmbH (Ed.) 8-Methoxypsoralen (Meladinine) Preliminary review of references Basotherm GmbH, D-7950 Biberach an der Riss 1. Postfach 130.

BECKER, S.W. (1960) Use and abuse of psoralens. *Journal of the American Medical Association*, **173**, 1483.

GRIFFIN, A.C. (1959) Methoxsalen in ultraviolet carcinogenesis in the mouse. *Journal of Investigative Dermatology*, **32**, 367.

HOPKINS, C.E., BELISARIO, J.C., MACDONALD, E.J. & DAVIS, C.T. (1963) Psoralen prophylaxis against skin cancer: report of clinical trial. II. *Journal of Investigative Dermatology*, 219.

JOSEPH, R., SHANTAMMA, M.S., REHANA, F. & NAND, K. (1974) Induced mutations in serratia by near uv-light in presence of psoralen. *Experientia*, **30**, 360.

JUHLIN, C. (1974) Effect of psoralens on psoriatic lesions. *First Dermatology Symposium of the Middle East Dermatological Society*, 1974. Cairo.

KAMMERAU, B., KLEBE, U., ZESCH, A. & SCHAEFER, H. (1975) Penetration, permeation, and resorption of 8-methoxypsoralen—comparative *in vitro* and *in vivo* studies after topical application of four standard preparations. *Archiv für Dermatologische Forschung*. In preparation.

LANGNER, A., WOLSKA, H., JARZABEK-CHORZELSKA, M. & PAWINSKA, M. (1975) Dermal toxicity of 8-methoxypsoralen in hairless mice irradiated with long uv. *Joint Meeting of the Society for Investigative Dermatology and the European Society for Dermatological Research, Amsterdam*, 1975.

MORTAZAWI, S.A.M. (1972) Meladinine und UVA bei Vitiligo, Psoriasis, Parapsoriasis und Akne vulgaris. *Dermatologische Wochenschrift*, **158**, 908.

MORTAZAWI, S.A.M. & OBERSTE-LEHN, H. (1973) Lichtsensibilisatoren und ihre therapeutischen Fähigkeiten. Erste vorläufige Mitteilung. *Zeitschrift für Haut- und Geschlechtskrankheiten*, **48**, 1.

O'NEAL, M.A. & GRIFFIN, A.C. (1957) The effect of oxypsoralen upon ultraviolet carcinogenesis in albino mice. *Cancer Research*, **17**, 911.

PARRISH, J.A., FITZPATRICK, T.B., TANENBAUM, C. & PATHAK, M.A. (1974) Photochemotherapy of psoriasis with oral methoxysalen and longwave ultraviolet light. *New England Journal of Medicine*, **291**, 1207.

SCHAEFER, H. & KUHN-BUSSIUS, H. (1970) Methodik zur quantitativen Bestimmung der menschlichen Talgsekretion. *Archiv für klinsche und experimentelle Dermatologie*, **238**, 429.

TOWNSEND, M.E., WRIGHT, H.M. & HOPWOOD, D.A. (1971) Efficient mutagenesis by near ultraviolet light in the presence of 8-methoxypsoralen in streptomyces. *Journal of Applied Bacteriology*, **34**, 799.

TRONNIER, H. & LOEHNING, R. (1974) About the current status of the methoxsalen-uv-A-therapy in dermatology. *Castellania*, **2**, 267.

TRONNIER, H. & LOEHNING, R. (1975) Photochemotherapie in der Dermatologie. *Deutsch-Schwedisches. Symposium: Photomedizin, Oberursel/Taunus, BRD*, 1975.

TRONNIER, H. & SCHÜLE, D. (1973) Zur dermatologischen Therapie von Dermatosen mit langwelligem UV nach Photosensibilisierung der Haut mit Methoxsalen. Erste Ergebnisse bei der Psoriasis vulgaris. *Zeitschrift für Haut- und Geschlechtskrankheiten*, **48**, 385.

URBACH, F. (1959) Modification of ultraviolet carcinogenesis by photoactive agents. Preliminary report. *Journal of Investigative Dermatology*, **32**, 373.

URBACH, F., FORBES, P.D. & DAVIES, R.E. (1975) On the relationship of phototoxicity to photocarcinogenesis. *Deutsch-Schwedsches Symposium: Photomedizin, Oberursel/Taunus, BRD*, 1975.

WALTER, J.F. & VOORHEES, J.J. (1973) Psoriasis improved by psoralen plus black light. *Acta dermato-venereologica*, **53**, 469.

WEBER, G. (1974a) Meladinine- und Blacklight-Behandlung der Psoriasis. 30*th Meeting of the Deutsche Dermatologische Gesellschaft in Graz*, 1974. *Archiv für dermatologische Forschung* (to be published).

WEBER, G. (1974b) Combined 8-methoxypsoralen and black-light therapy of psoriasis. Technique and results. *British Journal of Dermatology*, **90**, 317.

WEBER, G. (1974c) Blacklight-Behandlung der Psoriasis! Aufwand, Nutzen, Nachteile? *Dermatologische Mitteilungen*, **22**, 19.

WILLIS, I. & HARRIS, D.R. (1973) Resistant Psoriasis. *Archives of Dermatology*, **107**, 358.

WOLFF, K. (1975) *Deutsch-Schwedisches Symposium: Photomedizin, Oberursel/Taunus, BRD*, 1975.

WOLFF, K., PARRISH, J.A., FITZPATRICK, T.B., GSCHNAIT, F., GILCHREST, B., HONIGSMANN, H. & PATHAK, M.A. (1975) Photochemotherapy of psoriasis with oral psoralens. *Joint Meeting of the Society for Investigative Dermatology and the European Society for Dermatological Research, Amsterdam*, 1975.

British Journal of Dermatology (1976) **94**, 369.

Epidermal lipid metabolism in psoriasis and lichen simplex

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SUMMARY

The rate and pattern of epidermal lipogenesis from [^{14}C] glucose were measured in fifteen patients with psoriasis and three with lichen simplex, compared with twenty controls. In 'uninvolved' epidermis from psoriatic subjects the mean lipogenic rate was slightly raised, although the increase was not statistically significant. There was a positive correlation between overall lipogenic rate and the percentage of isotope appearing in free sterol, while the relative proportions of the other lipid classes were unchanged. By contrast, in control epidermis sterol percentage was negatively correlated with lipogenic rate.

In psoriatic lesions total epidermal lipogenesis (per unit surface area) was raised compared with matched control 'uninvolved' epidermis. Also raised were percentage labelling of free sterol and of combined (free sterol and monoesters), and the free sterol:monoester ratio was increased. Similar findings were obtained with lesions of lichen simplex, suggesting that disturbed sterol metabolism may be a common feature in conditions of abnormal keratinization.

The human epidermis is an active site of lipid biosynthesis (Hsia *et al.*, 1970; Summerly & Woodbury, 1971, 1972). For many years it has been suggested that sterol metabolism, in particular, is intimately connected with the process of keratinization (Yardley, 1969); increased ratios of free to esterified sterols have been reported in psoriatic lesions (von Glasenapp & Leonhardi, 1953) and scales (Wheatley & Farber, 1961; Wheatley, 1963) and in atopic eczema (Mustakallio *et al.*, 1967), and decreased cholesterol esterifying ability has been found on the lesion-free skin surface of psoriatic patients (Gara *et al.*, 1964).

More recently, changes in skin lipid biosynthesis have been reported in essential fatty acid deficiency, which is characterized in the rat by severe scaliness of the dorsal skin and extremities. Skin from these animals showed marked increases in the biosynthesis of neutral lipids, and especially sterol esters, and prostaglandin E_2 was effective both in inhibiting sterol ester biosynthesis and in clearing the scaly lesions (Ziboh & Hsia, 1972).

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In view of the possible connection between lipid biosynthesis and keratinization, it seemed of interest to examine the rate and pattern of epidermal lipogenesis in psoriasis and other disorders involving abnormal keratinization. In this paper we report studies on lesion and 'uninvolved' skin from fifteen patients with psoriasis and three with lichen simplex.

MATERIALS AND METHODS

The subjects for this study were fifteen volunteers (14 male and 1 female) with psoriasis, aged between 19 and 68 years, and three volunteers (2 male and 1 female), aged between 18 and 62 years, with lichen simplex. The control subjects were in two groups: group 1 comprised ten male subjects, aged between 17 and 61 years, with minor skin disorders as follows: grade 1 acne (five), alopecia areata (one), rosacea (one), mild malabsorption (one), leg ulcer (one) and warts (one). Group 2 comprised ten male subjects, aged between 16 and 37 years, with more severe acne (grades 2–4). No significant differences in epidermal lipogenesis were found between control groups 1 and 2, and the results for these groups have therefore been combined.

Skin biopsies were taken with a 4 mm punch, using a high-speed drill without local anaesthesia. Unless otherwise stated two biopsies were taken from the scapular region of the back, about 5 cm either side of the mid-line. In nine of the psoriatic patients one biopsy was taken from a lesion, and the other from lesion-free skin at a symmetrical site relative to the mid-line of the back. In patients with lichen simplex, biopsies of lesion and uninvolved skin were taken from sites on the leg.

Biopsies were rinsed in saline, and any subcutaneous adipose tissue was carefully removed. The method of measuring lipogenesis has been described in detail elsewhere (Cooper, Thody & Shuster, 1974). Briefly, the intact biopsies were incubated in 0·5 ml Krebs Ringer Bicarbonate containing benzylpenicillin (100 μg/ml), streptomycin sulphate (100 μg/ml) and 2 mM [U-^{14}C] glucose (5 mCi/mmol).

By using labelled glucose of relatively low specific activity, and in substrate quantities, it was hoped to achieve a steady state specific activity in the glucose pool and throughout the glycolytic and lipogenic pathways, and thus avoid the problems caused when a tracer such as acetate is used to label a pool of potentially variable size.

The incubation mixtures were gassed continuously with $O_2 + 5\%$ CO_2. Incubation was for 3 h, during which period incorporation of isotope into lipids is approximately linear in both rat skin (Cooper *et al.*, 1974) and human skin (unpublished observations). After incubation the tissue was removed, blotted, and placed in 0·5% acetic acid at room temperature for 2½–3 h. The epidermis could then be peeled off by gentle blunt dissection using small forceps and a No. 11 scalpel blade. Histological examination showed that this procedure removes epidermis together with some follicular mouths but that all sebaceous glands remain *in situ* in the dermis.

The discs of epidermis and dermis were extracted separately by the method of Bligh & Dyer (1959) and an aliquot of the total lipid extract was removed for scintillation counting. The remainder of the extract was analysed by one-dimensional thin-layer chromatography on silicic acid, together with standard carrier lipids to ensure resolution and visualization of the minor lipid components (Fig. 1). After location of the spots with iodine vapour, the plastic-backed plates were cut into sections and radioactivity was measured by liquid scintillation counting.

RESULTS

Rate and pattern of cutaneous lipogenesis in control subjects

In epidermis from control subjects, the mean incorporation of ^{14}C into total lipids was 4540±530

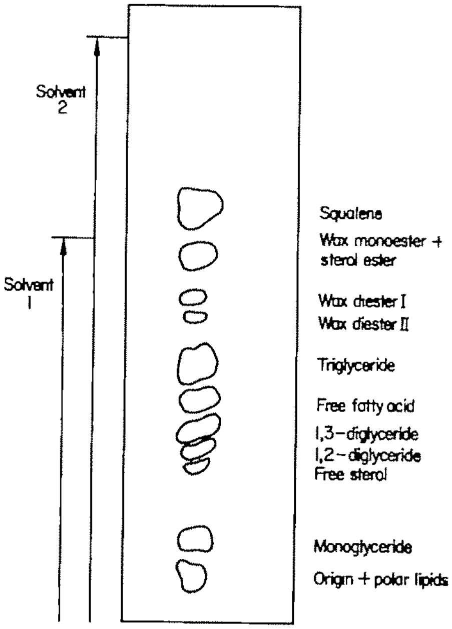

FIGURE 1. Thin-layer chromatography of carrier lipids on silicic acid using solvent systems as follows: Solvent 1, petroleum spirit (40–60°C)–diethyl ether–acetic acid (50:50:1); Solvent 2, petroleum spirit (40–60°C)–benzene (70:30).

dpm/biopsy/3 h (mean±s.e.m., n = 20), equivalent to 6·51±0·76 ng-atoms glucose carbon/cm^2/h. The bulk of this incorporation (58%) was into polar lipids, with smaller amounts of isotope appearing in free sterols, glycerides, free fatty acid, wax esters/sterol esters and squalene (Fig. 2).

For comparison, the pattern of dermal lipogenesis is shown for four of the control subjects (those without acne or rosacea). Incorporation of ^{14}C into total lipids averaged 70,580±7880 dpm/biopsy/3 h (mean±s.e.m., n = 4). Compared with epidermis, more isotope was present in triglyceride (55% of total) and in wax esters/sterol esters and squalene, with relatively less incorporation into free sterols and polar lipids (Fig. 2).

Lipogenesis in 'uninvolved' psoriatic skin

The rate of epidermal lipogenesis in lesion-free psoriatic skin, compared with control skin, is shown in Fig. 3. In psoriatic skin the mean ^{14}C incorporation was some 30% higher than in control skin, but this difference was not statistically significant.

When the pattern of lipogenesis was analysed it was found that, in psoriatic skin, the percentage of free sterol labelling relative to total lipids was increased at higher lipogenic rates ($r = 0{\cdot}679$, $P<0{\cdot}01$). The relative proportions of the other lipid classes were unchanged.

In skin from control subjects, the percentage of free sterol labelling decreased slightly at higher rates of lipogenesis ($r = -0{\cdot}258$). The control and psoriatic groups thus differed both in correlation coefficient ($P<0{\cdot}01$) and in regression coefficient (Fig. 4). These differences still remain if the point to the extreme right (Fig. 4) is excluded from the psoriatic group, although the significance level is not as high ($P<0{\cdot}05$).

In dermis of psoriatic skin, no significant correlation was apparent between percentage of free sterol labelling and overall lipogenic rate.

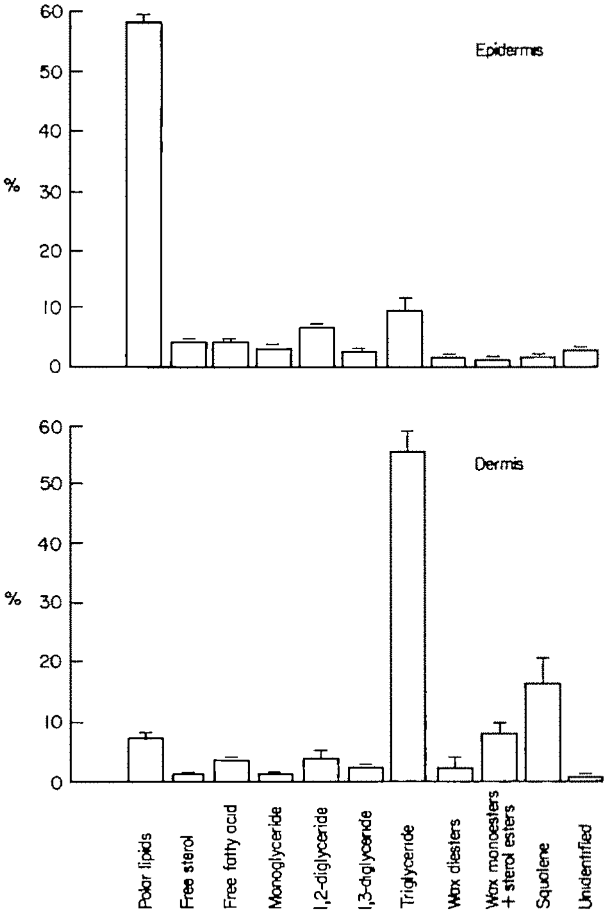

FIGURE 2. Patterns of lipogenesis in control epidermis (mean ± s.e.m., n = 20) and dermis (mean ± s.e.m., n = 4). Results are expressed as percentage of total isotope incorporated into lipids.

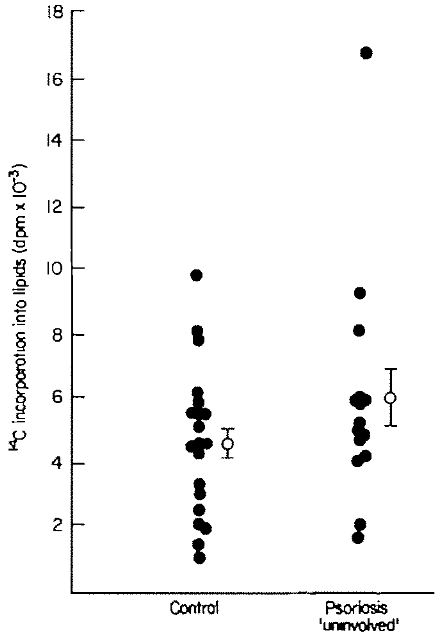

FIGURE 3. Rate of epidermal lipogenesis (per 4 mm biopsy) in control subjects and in 'uninvolved' skin from psoriatic subjects. Open circles and bars represent mean ± s.e.m. (n = 20, controls; n = 15, psoriatics).

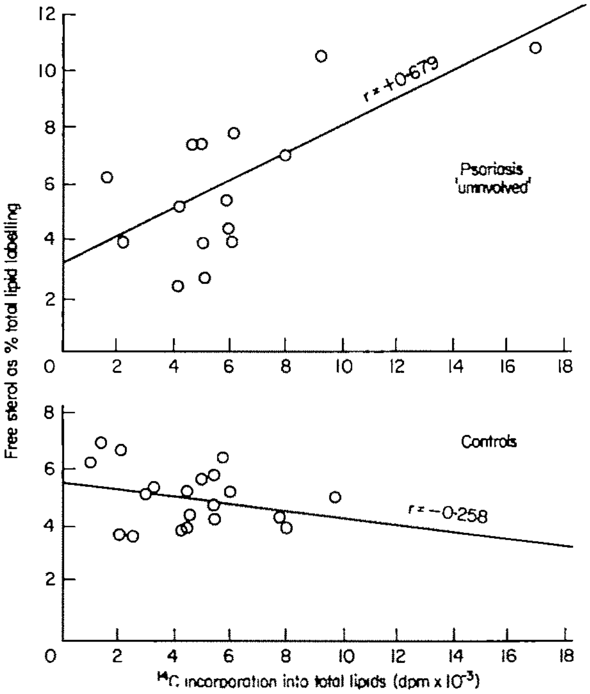

FIGURE 4. Relationship between free sterol labelling and total lipogenic rate in control and 'uninvolved' psoriatic epidermis. Sterol labelling is expressed as percentage of total isotope incorporated into lipids.

Lipogenesis in psoriatic lesions

In nine of the psoriatic patients, matched pairs of biopsies were taken from a lesion and from 'uninvolved' skin. All except one of the subjects had been undergoing treatment with dithranol (on lesions only) for varying periods of time; in the last subject (patient 9) the lesion site had received no treatment.

TABLE 1. Epidermal lipogenesis in 'uninvolved' skin and psoriatic lesions

Patient	Age	Sex	'Uninvolved' skin	Lesion
1	19	M	2030	4560
2	40	M	4680	22,180
3	64	M	5990	18,100
4	68	M	8050	17,980
5	43	M	5780	9980
6	29	M	4020	5750
7	51	M	5110	6870
8	46	M	1580	9720
9	26	F	4790	13,280

4 mm punch biopsies were incubated with [U-^{14}C] glucose as described in 'Methods'. Results are expressed as dpm incorporated into total epidermal lipids per biopsy per 3 h. $t_{\text{paired}} = 3·963$ ($P<0·005$).

Table 1 shows epidermal lipogenesis rates in 'uninvolved' skin and lesions. In each case the lipogenic rate was increased in the lesion ($P<0·005$), the increases ranging from 34 to 515%. The percentage of free sterol labelling was higher in the lesions ($P<0·001$, Table 2), and the percentage labelling of monoesters (i.e. the combined sterol ester and wax monoester fraction) was slightly

TABLE 2. Epidermal sterol biosynthesis in 'uninvolved' skin and psoriatic lesions

Patient	Free sterol (%)			Monoesters (%)			Ratio free sterol: monoesters	
	'Uninvolved'	Lesion	Change	'Uninvolved'	Lesion	Change	'Uninvolved'	Lesion
1	3·95	10·08	+6·13	1·34	0·61	−0·73	3·01	16·49
2	7·42	12·29	+4·87	1·30	0·45	−0·85	5·71	27·31
3	4·02	8·53	+4·51	3·89	3·80	−0·09	1·03	2·24
4	7·04	9·78	+2·74	0·66	0·46	−0·20	10·67	21·46
5	5·49	8·17	+2·68	0·28	0·24	−0·04	19·61	34·04
6	2·36	5·13	+2·77	0·38	0·42	+0·04	6·21	12·21
7	2·68	4·92	+2·24	1·42	0.66	−0·76	1·89	7·45
8	6·24	8·79	+2·55	2·05	0·48	−1·57	3·04	18·31
9	7·44	11·27	+3·83	1·44	0·55	−0·89	5·17	20·49
t_{paired}	8·108 ($P<0·001$)			3·190 ($P<0·02$)			5·504	($P<0·001$)

4 mm punch biopsies were incubated with [U-^{14}C] glucose as described in 'Methods'. Results are expressed as percentage of total isotope incorporated into epidermal lipids.

TABLE 3. Epidermal sterol biosynthesis in 'uninvolved' skin and psoriatic lesions

Patient	Free sterol dpm		Monoesters dpm	
	'Uninvolved'	Lesion	'Uninvolved'	Lesion
1	81	494	27	29
2	347	2726	61	101
3	241	1544	233	688
4	567	1758	53	82
5	315	815	16	24
6	95	295	15	24
7	137	338	72	45
8	99	854	32	47
9	356	1497	69	73
t_{paired}	$3{\cdot}864$ ($P<0{\cdot}005$)		$1{\cdot}192$ (NS)	

4 mm punch biopsies were incubated with [U-^{14}C] glucose as described in 'Methods'. Results are expressed as dpm incorporated into sterol or monoesters per biopsy.

decreased ($P<0{\cdot}02$, Table 2); thus the ratio of free sterol to monoesters was raised ($P<0{\cdot}001$, Table 2). In absolute terms the labelling of both these lipid classes was increased, but monoesters not significantly so (Table 3). Overall, the percentage of free sterol+monoesters, relative to total lipids, was higher in lesion then in uninvolved skin (from results in Table 2).

When free sterol percentage was plotted against total lipogenic rate in the lesions, there was a positive correlation ($r = 0{\cdot}625$, $0{\cdot}1 > P > 0{\cdot}05$) and the points fitted on the regression line obtained with 'uninvolved' skin (Fig. 5).

In dermis from lesions, neither lipogenesis rate nor free sterol percentage were significantly different from the matched controls.

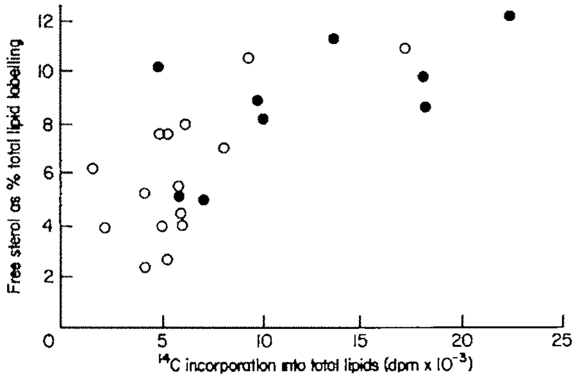

FIGURE 5. Relationship between free sterol labelling and total lipogenic rate in epidermis from 'uninvolved' skin (○) and psoriatic lesions (●). Sterol labelling is expressed as percentage of total isotope incorporated into lipids.

Lipogenesis in lichen simplex

Epidermal lipogenesis was also studied in three patients with lichen simplex. In each case total lipogenic rate was increased in the lesions (Table 4), and both percentage of free sterol, and ratio of free sterol to monoesters, were increased (Table 5).

TABLE 4. Epidermal lipogenesis in 'uninvolved' skin and lesions of lichen simplex

Patient	Age	Sex	'Uninvolved' skin	Lesion
1	18	M	1750	16,510
2	36	M	7390	9990
3	62	F	7640	11,910

4 mm punch biopsies from the tibial region (patients 1 and 2) or thigh (patient 3) were incubated with [U-^{14}C] glucose as described in 'Methods'. Results are expressed as dpm incorporated into total epidermal lipids per biopsy per 3 h.

TABLE 5. Epidermal sterol biosynthesis in 'uninvolved' skin and lesions of lichen simplex

Patient	Free sterol (%)		(Free sterol + monoesters) (%)		Ratio free sterol: monoesters	
	'Uninvolved'	Lesion	'Uninvolved'	Lesion	'Uninvolved'	Lesion
1	7·07	12·19	7·98	12·48	7·77	42·03
2	3·85	7·03	4·14	7·33	13·28	23·43
3	3·74	5·81	4·06	6·29	11·69	12·10

4 mm punch biopsies were incubated with [U-^{14}C] glucose as described in 'Methods'. Results are expressed as percentage of total isotope incorporated into epidermal lipids.

DISCUSSION

The results presented here confirm earlier reports of increased sterol and fatty acid biosynthesis in psoriatic lesions (Herdenstam, 1962; Aso *et al.*, 1971) and indicate that the increased lipogenesis is confined to the epidermis.

The possibility that the differences seen here in lipid labelling result from alterations in specific activity of precursor pools, cannot entirely be ruled out. The method we use was designed to minimize this possibility by using a labelled precursor in substrate quantities as the only exogenous carbon source (Cooper *et al.*, 1974). Wheatley *et al.* (1971) have shown that glucose utilization for lipid synthesis in skin approximately equals the absolute rate of lipogenesis, as measured by ^3H_2O incorporation, and it therefore seems likely that lipid labelling from glucose in the present experiments parallels the absolute rate of lipogenesis.

Most of the psoriatic patients in this study had started dithranol treatment, but as similar changes were seen in the single untreated patient with psoriasis and in the patients with lichen simplex the increases in lipogenesis could not be explained by the treatment. The possibility that larger changes occur in untreated lesions will require further investigation.

No attempt has been made to relate epidermal lipogenesis rates to weight of tissue, as the epidermal separation and extraction methods used do not permit an accurate measurement of either wet or dry weight. Thus it is not possible to say how far the increases in lipogenesis which we found in the lesions are related to the increased epidermal thickness. Herdenstam's findings (Herdenstam, 1962) of an increase in lipogenesis per unit wet weight of tissue, could mean that the changes in psoriatic lesions are not simply a consequence of increased epidermal thickness. The question must remain open, however, as Herdenstam's use of dermatome slices would give a greater contribution of dermal weight in the lesion-free tissue.

The marked increases in free sterol biosynthesis, relative to other lipids, in psoriatic lesions (Table 2) are consistent with previous reports of an increased free sterol:sterol ester ratio in lesions and scales. The increases in free sterol percentage far outweighed the decreases seen in the percentage of monoesters (Table 2), and in absolute terms the labelling of both lipid fractions was increased, but free sterol much more than monoesters (Table 3). Thus, quantitatively, the largest contributing factor to the altered sterol:sterol ester ratio would seem to be an acceleration of sterol biosynthesis, rather than a reduction in sterol esterification. This interpretation does not, however, preclude the possibility that under normal conditions sterol esters could act as end-product inhibitors of sterol biosynthesis, so that a block in sterol ester production could lead to higher rates of free sterol biosynthesis. This would be consistent with the observation of decreased cholesterol esterifying ability on the lesion-free skin surface of psoriatic subjects (Gara *et al.*, 1964).

The finding of a positive correlation between total lipogenic activity and percentage of label appearing in free sterol, in both lesion and uninvolved psoriatic skin (Fig. 5), but not in control skin (Fig. 4), is suggestive of a qualitative abnormality in epidermal lipid metabolism in psoriasis. This possibility will require further detailed study for confirmation. Reinertson & Wheatley (1959) found no overall differences in lipid and sterol content between normal and uninvolved psoriatic epidermis, and the question whether surface lipid differs has been a matter for some dispute (Rothman, 1950; Konopik *et al.*, 1966; Wilkinson & Farber, 1967a, b). Until further direct information from metabolic studies is available, the possibility of a disturbance in lipid metabolism in 'uninvolved' psoriatic epidermis must remain an open question.

The increases in free sterol biosynthesis in psoriatic lesions (Table 2) stand in marked contrast to the results of Ziboh & Hsia (1972) for essential fatty acid-deficient skin. These workers found large increases in sterol ester biosynthesis, with only a minor change in free sterol labelling. Thus, although a disturbance of sterol metabolism is found in both psoriasis and EFA deficiency, the nature of the disturbance in the two cases is fundamentally different. This may indicate that for normal keratinization to occur, it is necessary to maintain a correct balance between rates of sterol biosynthesis and esterification, and that a disturbance of this balance in either direction could lead to disruption of the keratinization process.

The question arises whether sterol metabolism is altered in other conditions where abnormal keratinization occurs. Summerly & Yardley (1967) found increased sterol biosynthesis in epidermis from patients with ichthyosis vulgaris, compared with normal controls. Although this increase was not statistically significant, the authors pointed out that the ichthyotic specimens contained metabolically inert scale, and so the true increase in sterol synthesis per unit of viable tissue was probably greater. In the present paper, results for lichen lesions show changes virtually identical to those found in psoriasis (Tables 4 and 5). This suggests that increased sterol biosynthesis may be a common feature of disorders of epidermal hyperplasia and hyperkeratinization. Further comparative studies will be necessary to establish whether a disturbance in sterol metabolism is consistently found associated with such conditions.

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REFERENCES

Aso, K., Fujita, M., Okazaki, T. & Katoh, T. (1971) Studies on the lipogenesis of psoriatic epidermis. *Japanese Journal of Dermatology, Series B*, **81**, 161.

Bligh, E.G. & Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry*, **37**, 911.

Cooper, M.F., Thody, A.J. & Shuster, S. (1974) Hormonal regulation of cutaneous lipogenesis: effects of hypophysectomy, posterior hypophysectomy and α-melanocyte-stimulating hormone treatment. *Biochimica et Biophysica Acta*, **360**, 193.

Gara, A., Estrada, E., Rothman, S. & Lorincz, A.L. (1964) Deficient cholesterol esterifying ability of lesion-free skin surfaces in psoriatic individuals. *Journal of Investigative Dermatology*, **43**, 559.

Glasenapp, I. von & Leonhardi, G. (1953) Uber den Cholesterinstoffwechsel in der Gesunden und der psoriatisch veranderten Haut. *Archiv für Dermatologie and Syphilologie*, **96**, 148.

Herdenstam, C.-G. (1962) On the *in vitro* metabolism of labelled glucose in normal and psoriatic skin slices. *Acta dermato-venereologica*, **42**, suppl. 47.

Hsia, S.L., Fulton, J.F. Jr., Fulghum, D. & Buch, M.M. (1970) Lipid synthesis from acetate-1-^{14}C by suction blister epidermis and other skin components. *Proceedings of the Society for Experimental Biology and Medicine*, **135**, 285.

Konopik, J., Zaruba, F., Belsan, I., Krs, C., Zvereva, E. & Spanlangova, I. (1966) Zur Diagnose der latenten Psoriasis. *Archiv für Klinische und experimentelle Dermatologie*, **227**, 841.

Mustakallio, K.K., Kiistala, U., Piha, H.J. & Nieminen, E. (1967) Epidermal lipids in Besnier's Prurigo (atopic eczema). *Annales Medicinae Experimentalis et Biologiae Fenniae*, **45**, 323.

Reinertson, R.P. & Wheatley, V.R. (1959) Studies on the chemical composition of human epidermal lipids. *Journal of Investigative Dermatology*, **32**, 49.

Rothman, S. (1950) Abnormalities in the chemical composition of the skin surface film in psoriasis. *Archives of Dermatology and Syphilology*, **62**, 814.

Summerly, R. & Woodbury, S. (1971) The *in vitro* incorporation of ^{14}C-acetate into the isolated sebaceous glands and appendage freed epidermis of human skin. *British Journal of Dermatology*, **85**, 424.

Summerly, R. & Woodbury, S. (1972) Lipid synthesis (^{14}C-acetate incorporation) in the isolated human sebaceous gland, the appendage-freed epidermis, the sebocyst (steatocystoma) and the wen (keratinous cyst of skin). *British Journal of Dermatology*, **86**, 614.

Summerly, R. & Yardley, H.J. (1967) Cholesterol synthesis in ichthyosis vulgaris. *British Journal of Dermatology*, **79**, 378.

Wheatley, V.R. (1963) The lipid components of the psoriatic skin. In: *Proceedings of the XII International Congress of Dermatology* (Ed. by D.M.Pillsbury and C.S.Livingood), Vol. 1, p. 157. Excerpta Medica Foundation, Amsterdam.

Wheatley, V.R. & Farber, E.M. (1961) Studies on the chemical composition of psoriatic scales. *Journal of Investigative Dermatology*, **36**, 199.

Wheatley, V.R., Hodgins, L.T., Coon, W.M., Kumarasiri, M., Berenzweig, H. & Feinstein, J.M. (1971) Cutaneous lipogenesis: precursors utilized by guinea-pig skin for lipid synthesis. *Journal of Lipid Research*, **12**, 347.

Wilkinson, D.I. & Farber, E.M. (1967a) Free and esterified sterols in surface lipids from uninvolved skin in psoriasis. *Journal of Investigative Dermatology*, **48**, 249.

Wilkinson, D.I. & Farber, E.M. (1967b) Fatty acids of surface lipids from uninvolved skin in psoriasis. *Journal of Investigative Dermatology*, **49**, 526.

Yardley, H.J. (1969) Sterols and Keratinization. *British Journal of Dermatology*, **81**, Suppl. 2, 29.

Ziboh, V.A. & Hsia, S.L. (1972) Effects of prostaglandin E_2 on rat skin: inhibition of sterol ester biosynthesis and clearing of scaly lesions in essential fatty acid deficiency. *Journal of Lipid Research*, **13**, 458.

British Journal of Dermatology (1976) **94**, 379.

The natural history of trichilemmal cysts

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SUMMARY

The histological features of 149 trichilemmal cysts removed from sixty-five patients are reviewed and related to the clinical findings. These cysts, which may be solitary or multiple, gradually increase in size and number, and may produce daughter cysts by budding. When a breach occurs in the wall, inflammatory cells pour in but do not replace the cyst wall. This event may be followed by healing from the margins of the breach, by marsupialization to the overlying epidermis and thus natural resolution, or by proliferation to produce a pseudo-epitheliomatous change which can be confused with a well differentiated squamous cell carcinoma.

We are here describing the natural history of the common wen, which many clinicians and pathologists call the sebaceous cyst. Its histogenesis is more accurately defined by the title trichilemmal cyst (Pinkus, 1969). This work began as an analysis of the clinical, histological and genetic features of trichilemmal cysts and has been reported in some detail elsewhere (Leppard, Sanderson & Wells, 1976). The large number of excised cysts provided by the study has thrown light on the evolution of the lesion and its responses to injury and inflammation. We consider the findings are worthy of a separate communication because of the unexpected features seen within the cyst walls.

MATERIALS AND METHODS

One hundred and fifteen patients with trichilemmal cysts were studied. These were a series derived from sixty probands and their families seen at one hospital. When an individual was first seen a full clinical history and examination were carried out. Any cysts which were present were removed. The specimens were embedded in paraffin, sectioned at 5 μm and stained with haematoxylin and eosin, periodic acid—Schiff, and by the von Kossa method. After the sixty probands had been seen, as many as possible of their relatives were seen and examined in the same way. Only five relatives agreed to have cysts removed for histological examination. One hundred and forty nine cysts were available for histological studies from these sixty-five patients, and they were not selected in any way to give a bias in favour of unusual histological changes.

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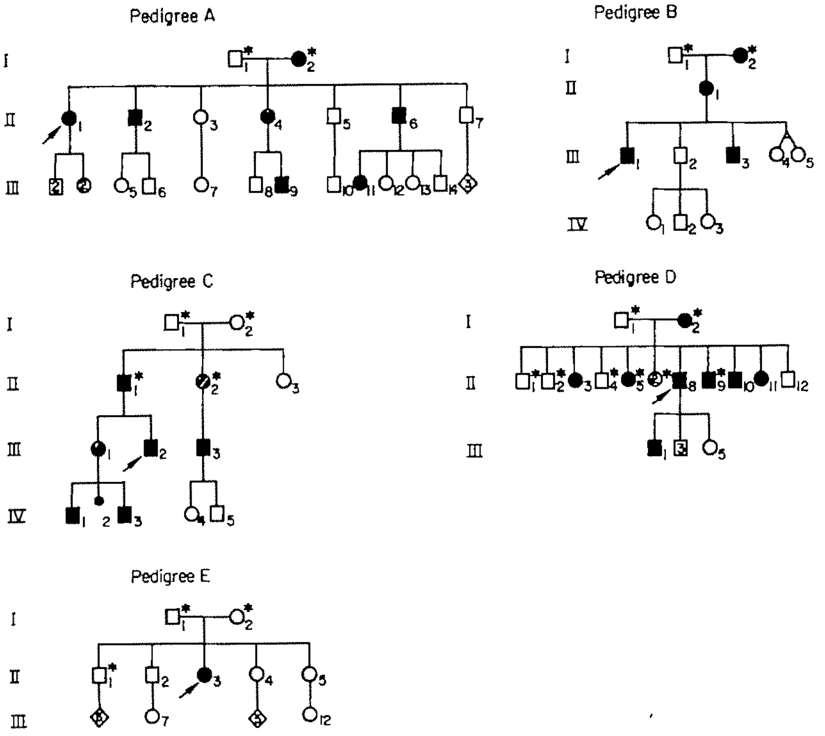
RESULTS

Clinical and genetic features

Trichilemmal cysts occur most commonly on the scalp, but can be found on all other parts of the body except the palms, soles, genitalia, axillae and groins. They can be differentiated from epidermoid cysts and steatocystoma multiplex by histological examination. The following family histories illustrate many of their features and the autosomal dominant mode of inheritance.

Pedigree A. Mrs V.A., a 56-year-old female, II.1, noticed a lump on the front of her scalp at the age of 18 years. Twelve years later she 'picked it' and the cyst discharged a white cheesy material and became much smaller. One year ago she caught it in her comb and it discharged again, and has continued to do so since then (Fig. 2). It was excised and shown histologically to be a marsupialized trichilemmal cyst. She had three other ordinary trichilemmal cysts, two on the scalp and one on the back of her right thigh. The other affected members of her family all had several cysts on their scalps.

Pedigree B. The proband, III.1, and his younger brother III.3, both had several cysts on the scalp. Their mother, II.1. had a solitary cyst on the top of her head which she scratched with a comb 1 year ago. Following this it developed a small extrusion on top which then burst. The cyst discharged for several months, leaving a hard crust on top which eventually dropped off leaving no sign of any cyst.



■● Male/female affected; ▨⊘ Male/female probably affected; □○ Male/female unaffected,

□*○* Male/female deceased, Monozygotic twins; • Miscarriage; ↗ Proband.

FIGURE 1.

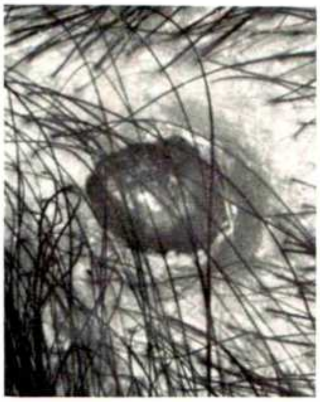


FIGURE 2. Marsupialized trichilemmal cyst (Pedigree A, II.1).

The grandmother, I.2, had had three large cysts on the scalp, one of which opened of its own accord and disappeared.

Pedigree C. The proband, III.2, had a large cyst on his right lower back formerly thought to be a lipoma. On histological examination it proved to be a proliferating trichilemmal cyst. He had seven other cysts, five on the scalp and two on the trunk which were ordinary trichilemmal cysts.
This family has already been reported in more detail (Leppard *et al.*, 1976, Pedigree B).

Pedigree D. The proband, II.8, a 54-year-old Hungarian, began to develop cysts on his scalp at the age of 23 years. Fifteen cysts were present when he was seen by us and twelve of these were removed

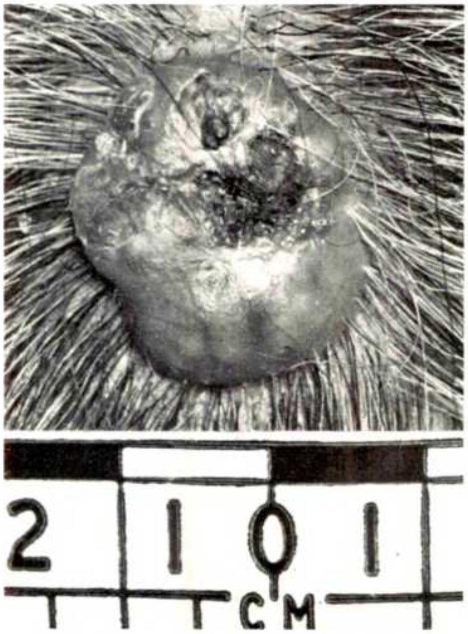


FIGURE 3. Proliferating trichilemmal cyst (Pedigree E, II.3).

for histological study. One showed early proliferative changes. His 25-year-old son had three cysts on his scalp, and five of the proband's eleven siblings also had scalp cysts.

Pedigree E. A 60-year-old mentally retarded schizophrenic female, II.3, had a lump on the top of her scalp for the last year. She had recently knocked it and it had increased in size and become crusted (Fig. 3). It was removed under local anaesthesia and shown to be a proliferating trichilemmal cyst. She had no other cysts and no other members of her family were affected as far as was known.

The clinical features of trichilemmal cysts are thus relatively straightforward. Patients present themselves with smooth, rounded, intradermal swellings which, in 89% of individuals, are on the scalp. They may be solitary (30%) or multiple (70%) and gradually increase in size. Some individuals develop increasing numbers with time, but it is unusual to find very large numbers of cysts, for example only 10% of the patients had more than ten cysts. Usually, multiple cysts on the scalp are of multicentric origin occurring all over the scalp. They may, however, all come from one cyst and appear as recurrences following removal of a cyst, or several cysts may be found when an apparently solitary cyst is removed. Two other changes which seem to be peculiar to trichilemmal cysts are marsupialization and proliferation.

In this study a marsupialized cyst was seen clinically in two female patients (Pedigree A, II.1; Pedigree B, II.1), one of whom had other trichilemmal cysts in addition. It may occur more often than this because a history of cysts disappearing on their own was given by eleven other females (no males). Since this study was completed 2 years ago, three other females with marsupialized cysts have been seen by one of us (B.J.L.). When a cyst marsupializes it may look like a small hole on the scalp discharging keratin or it may have a crust on top (Fig. 2). Because there is now an opening into the cyst from the outside it may become infected and inflamed.

Three patients (2%) had proliferating cysts, two males (Pedigree C, III.2; Pedigree D, II.8) and one female (Pedigree E, II.3). Their ages were 59, 51 and 59 years respectively, but there was no way of knowing exactly how long any of these cysts had been present. Two patients had multiple trichilemmal cysts and one had only the proliferating cyst. Initially this may look no different from an ordinary trichilemmal cyst and the proliferation may simply be an incidental histological finding, or it may look like a 'tumour'. The proliferating cyst of the proband in Pedigree E had the clinical appearance of a cylindroma (Fig. 3); the other two were incidental histological findings.

The patient's history was not a reliable means of forecasting the histological changes (Table 1). Cysts which suddenly enlarged were proliferating in two patients, but no reason was found in seven others. Cysts which were said to be infected were marsupialized (2), had disappeared on their own (5) and thus can be presumed to have been previously marsupialized, or were found to have proliferated (2).

TABLE 1. Clinical history of 115 patients with trichilemmal cysts from sixty families

Clinical history	No. of patients	%
Cysts increased in size with time	76	66·1
Cysts increased in number with time	59	51·3
Cysts suddenly enlarged	9	7·8
Cysts have been infected	17	14·8
Cysts have disappeared on their own	11	9·6

Histological features

One hundred and forty-nine cysts from sixty-five patients were examined, fourteen cysts from one patient, twelve cysts from two patients, six from one patient, five from three patients, four from four patients, three from three patients, two from fourteen patients and one from thirty-seven patients. One hundred and eleven (74·5%) were straightforward trichilemmal cysts with the characteristic lining of pale cells, increasing in vertical diameter as they mature and changing abruptly into solid, eosinophilic-staining keratin without the formation of a granular layer. Eleven cysts (7·4%) showed some evidence of keratohyalin formation in the walls and the remains of nucleated cells within the central keratin. Cholesterol clefts were present in 133 cysts (89%) and 36 cysts (24%) were calcified, this being present within the keratin, usually in large clumps or in a linear pattern between the laminated keratin. Cysts which were calcified were otherwise no different from those which were not with regard to size or age of the cysts. Calcification was thus not confined to 'old' cysts. Ninety-two other cysts showed a bluish discoloration of the central keratin when stained with haematoxylin and eosin. This was originally thought to be due to calcium, but the bluish areas did not react with the von Kossa stain. We now think that this phenomenon was due to a degenerative change of the keratin. One cyst showed a telogen hair root attached to the wall of the cyst and in another section, a similar telogen hair was cut in cross section and attached to the cyst, both being enclosed within the vitreous membrane.

Two cysts showed areas of focal maturation within the wall (Fig. 4), reminiscent of the changes that occur within seborrhoeic warts. This change might be the first stage of budding from the wall which was seen in four (2·7%) of the 149 cysts. Some cysts showed evidence of several cysts budding off from one parent cyst (Fig. 5) and one cyst had 12 new cysts coming from it. In one cyst budding had started around a plaque of calcium (Fig. 6) with the lining cells growing down into the cyst around the calcium. If this area were to separate, it would give a new cyst that was already calcified.

Inflammatory changes within the wall of a trichilemmal cyst differed from those in epidermoid cysts and were relatively less common. Only eighteen cysts (12%) showed inflammatory changes, in every case in an area where there was a break in the cyst wall (Fig. 7). This was often at a place where plaques of calcium were closely applied to the cyst wall. Instead of the inflammatory cells replacing the cyst wall with granulation tissue as occurs in epidermoid cysts, the break in the wall allows the entrance of lymphocytes, histiocytes, giant cells, fibroblasts and small blood vessels which begin to organize. In such an area, the vitreous membrane has disappeared and the inflammatory cells are associated with cholesterol clefts and plaques of calcium. These cysts are the ones which are likely to 'burst' when they are being removed; cysts without this change can usually be removed intact because they separate from the surrounding vitreous membrane.

There may be three sequels to this inpouring of inflammatory tissue. First, the breach in the wall may be repaired by epiboly from the margins. Eventually a new cyst wall may be produced which is nearly indistinguishable from one that has not been damaged, the only clue being that there are small capillaries and fibroblasts within the keratin of the cyst. Second, the cyst wall on either side of the breach may join the overlying epidermis to give a marsupialized cyst, which discharges its contents onto the surface. Third, the lining cells of the cyst may increase in number and thicken in the area of the inflammatory tissue and begin to enclose it. This may be the first stage of proliferation, which progresses to irregular hyperplasia (Fig. 8). True proliferation may then ensue to produce a pseudo-epitheliomatous appearance, which has often in the past been mistaken for a squamous cell carcinoma. Three of the 149 cysts examined showed this proliferating change and all three showed part of an ordinary trichilemmal cyst at one end. The histological changes seen in the proliferating cyst were very variable. The tumours were lobulated, but nevertheless well circumscribed, being divided up by connective tissue septa and inflammatory cells (Fig. 9). In some areas, masses of pale-staining, glycogen-filled cells were present, which were no different from the cyst lining cells, except that there were

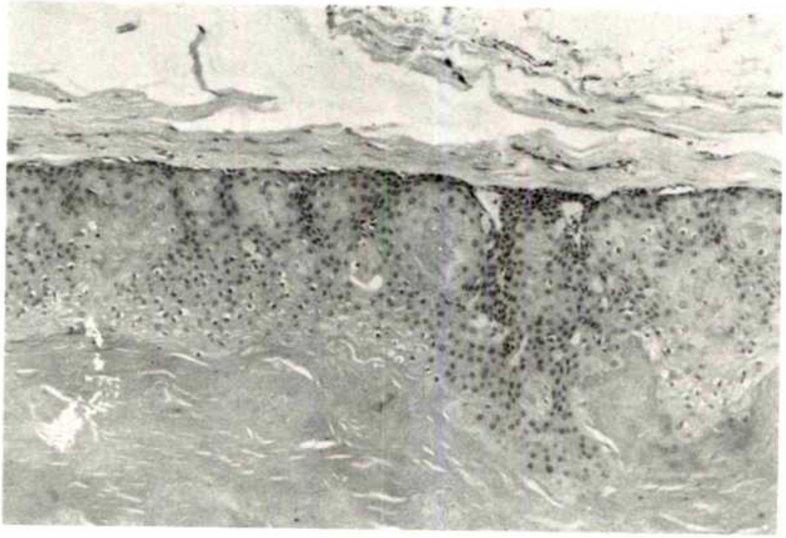


FIGURE 4. Focal areas of maturation within cyst wall (H & E, × 100).

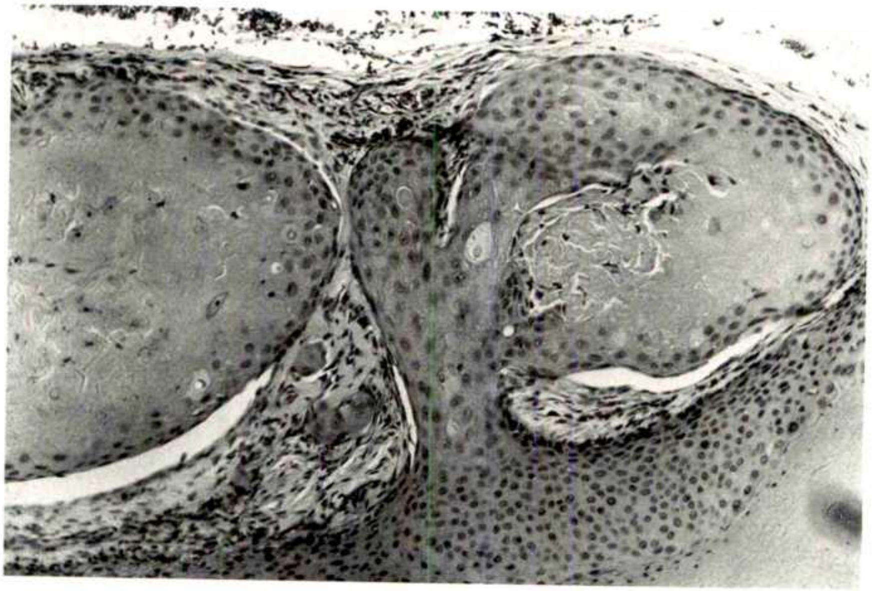


FIGURE 5. Budding from cyst wall (H & E, × 144).

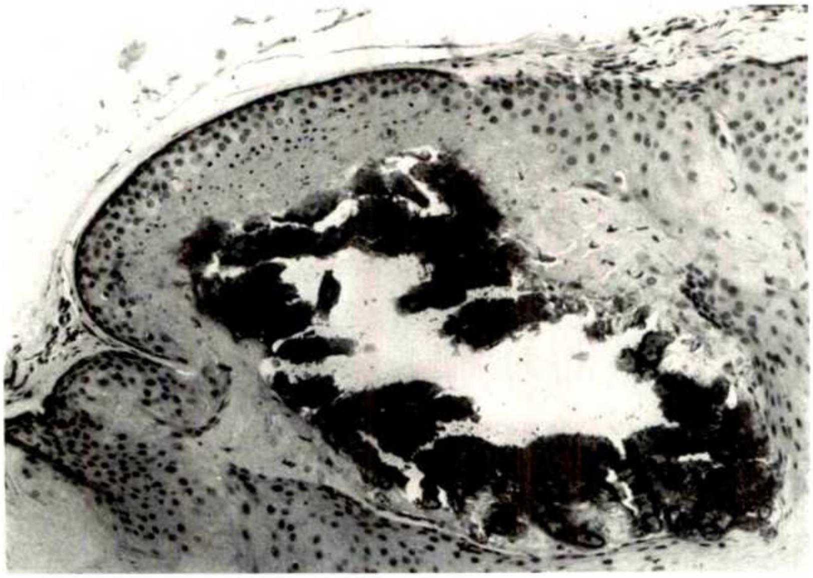


FIGURE 6. Budding around a plaque of calcium (H & E, × 144).

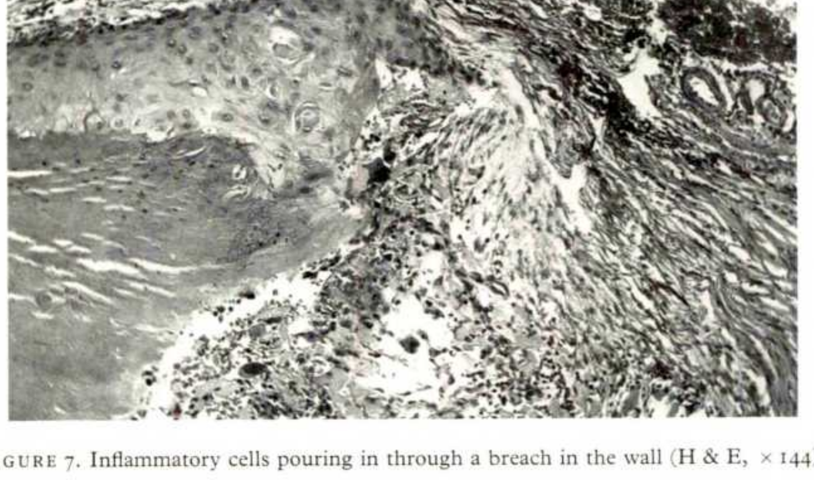


FIGURE 7. Inflammatory cells pouring in through a breach in the wall (H & E, × 144).

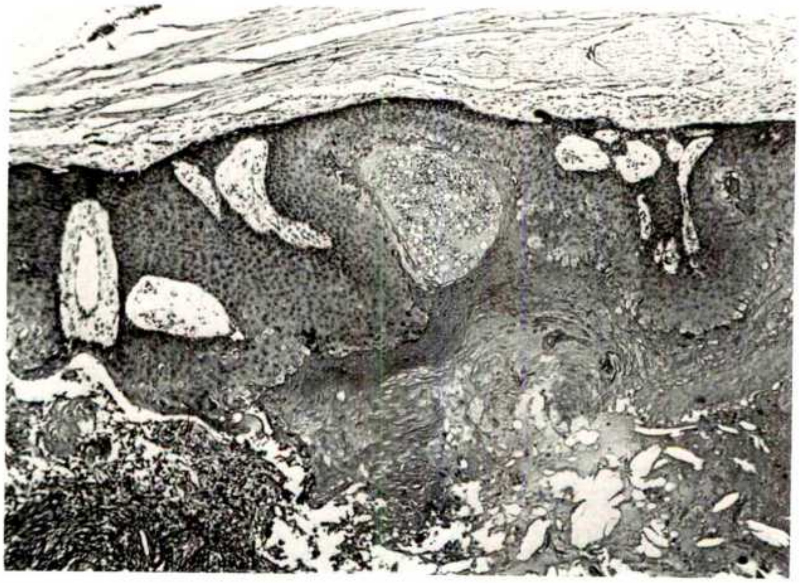


FIGURE 8. Early proliferation of trichilemmal cyst (H & E, × 54).

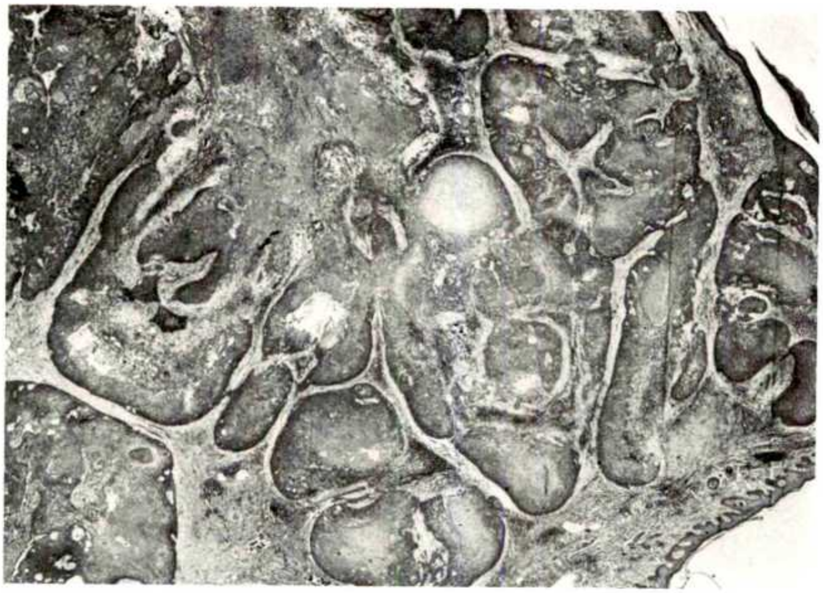


FIGURE 9. General architecture of proliferating trichilemmal cyst. Tumour lobules are separated by connective tissue septae and inflammatory cells (H & E, × 15).

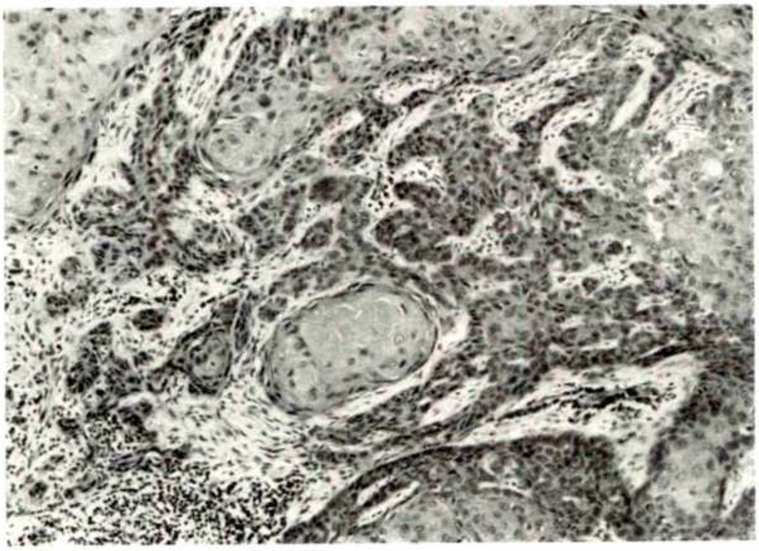


FIGURE 10. Proliferating trichilemmal cyst (H & E, × 100).

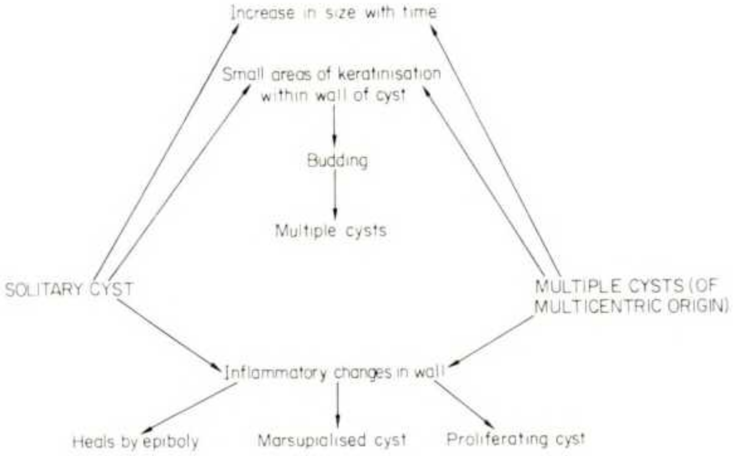

Increase in size with time
Small areas of keratinisation within wall of cyst
Budding
Multiple cysts
SOLITARY CYST
MULTIPLE CYSTS (OF MULTICENTRIC ORIGIN)
Inflammatory changes in wall
Heals by epiboly
Marsupialised cyst
Proliferating cyst

FIGURE 11.

more of them, and some groups of these cells had central foci of keratinization. Other areas showed dyskeratosis with larger, irregular shaped cells with hyperchromatic nuclei which looked very like a squamous cell carcinoma (Fig. 10). Inflammatory cells, mostly lymphocytes, histiocytes and giant cells were seen between the tumour lobules together with cholesterol clefts, calcium and new blood vessel formation. These histological changes are summarized in Fig. 11.

DISCUSSION

It is important to distinguish the three main types of skin cyst from each other, because their genesis and natural history are different. Epidermoid cysts commonly follow damage to the pilosebaceous follicle or implantation of epidermis, but may also be a component of several genetically determined syndromes such as Gardner's syndrome (Leppard & Bussey, 1975). Trichilemmal cysts and steatocystoma multiplex are both genetically determined structural aberrations of the pilosebaceous follicle, inherited as specific entities and not as mixtures with other types of cyst (Leppard *et al.*, 1976).

There is no experimental work on the origin of trichilemmal cysts. They have not been produced, for example, by implantation of skin in humans (Epstein & Kligman, 1957) or in animals (Schweninger, 1884; Baker & Mitchell, 1965). There is no animal model which produces them naturally in the way that homologous recessive hairless mice form epidermoid cysts (Montagna, Chase & Melaragno, 1952). Nor has the relationship to the adjacent follicles been traced by serial section as has been done with steatocystoma multiplex (Kligman & Kirschbaum, 1964). It seems likely that they are produced by 'budding off' from the external root sheath in the region of the follicular isthmus, in the same way that new cysts bud from a parent cyst. There is no conclusive evidence that this occurs, but our observation of a cyst attached to a telogen hair root supports the idea.

Individual cysts grow progressively and some of the central keratin may disintegrate to produce a pseudo-calcareous mass which causes softening of the cyst when palpated. True calcification is found in nearly one-quarter of all cysts regardless of their size or age. The cholesterol clefts which are almost universally present derive from lipids produced by the lining cells of the cyst (Nicolaides, Levan & Fu, 1968). Budding from a trichilemmal cyst may occur *de novo* from foci of maturation in the cyst wall. These areas are then pushed out to produce daughter cysts. This is one reason for the tendency for trichilemmal cysts to recur after excision, when only the parent cyst is removed and one or more small daughter cysts are left behind. Budding may also occur directly around areas of calcification, producing small daughter cysts which are calcified from the beginning.

The response of these cysts to inflammation is perhaps their most remarkable and distinctive quality. Clinically, trichilemmal cysts become inflamed infrequently; 14·8% in this series compared with 50% of epidermoid cysts (Leppard, 1976). Such inflammatory changes as do occur are not due to bacterial infection as might be expected. There is normally no canal from the cyst to the surface, no bacteria are seen inside any of the cysts microscopically and they cannot be grown on culture of the contents (Noble, Leppard & Thomson, 1976).

When the wall of a cyst and its enclosing vitreous membrane break and allow the keratinous contents to come into contact with the surrounding mesodermal structures, inflammatory cells, fibroblasts and capillaries enter through the hole. The response of the wall to such an invasion is either simple repair by growth from the contiguous margins, marsupialization and later disappearance of the cyst, or proliferation to produce a pseudo-epitheliomatous mass.

When simple repair takes place, the patient may be unaware that anything has happened and the only evidence is the persistence of mesodermal elements within the apparently intact cyst cavity. When a cyst marsupializes, its contents are discharged on to the surface and the cyst gradually decreases in size until it disappears. It would seem that this is the way that cysts can disappear on their own. However, whilst open to the surface they may become secondarily infected. Both patients with marsupialized cysts said that their cysts were 'infected', and they were in fact clinically inflamed. This change is probably quite common, and from our patients' histories seems more likely to occur in women, possibly after injury to the cyst.

The proliferating change is relatively uncommon, being seen in only 2% of cysts in this study, but the early stages (Fig. 7) may easily be missed when reviewing the microscopic features of trichilemmal cysts, and large numbers of cysts are not sent for histological examination. Whether a true squamous cell carcinoma ever arises from a trichilemmal cyst or a proliferating cyst is unknown. Local recurrence after excision of a proliferating cyst has been reported (Seff & Berkowitz, 1916; Wilson Jones, 1969), and so has lymph node involvement (Peden, 1948; Holmes, 1968), although in the last two reports the patients were still alive and well 11 years and 5 years respectively after histologically proven lymph node involvement.

There has been much speculation about the cause of the proliferative change, and suggestions include irritation from the contents of the cyst (Puhr, 1934), infection (Bishop, 1931; Nürnberger, 1938; Muehlon, 1942; Lever & Griesemer, 1949), and trauma. It is unlikely that infection plays a part, because trichilemmal cysts do not usually become infected (Noble *et al.*, 1975). Epidermoid cysts, which frequently become infected, have not been reported to undergo proliferation, and the authors have never seen this change in such cysts. The ingrowth of granulation tissue into the cyst cavity places the epithelial wall in a peculiar situation, sandwiching it between mesodermal elements; this seems to be the vital initiating factor which persists even in the grossly proliferating lesions (Fig. 9). It is likely that local trauma plays a part in the initial breach in the cyst wall but why some cysts heal, others marsupialize and yet others proliferate cannot be explained.

ACKNOWLEDGMENTS

We would like to thank the Surgeons at St George's Hospital for allowing us to see their patients with cysts, our Dermatological colleagues for referring their patients to us and the members of the photographic department at St John's Hospital for considerable help.

REFERENCES

Baker, B.R. & Mitchell, D.F. (1965) The pathogenesis of epidermoid implantation cysts. *Oral Surgery, Oral Medicine and Oral Pathology*, **19**, 494.

Bishop, E.L. (1931) Epidermoid carcinoma in sebaceous cysts. *Annals of Surgery*, **93**, 109.

Epstein, W.L. & Kligman, A.M. (1957) Epithelial cysts in buried human skin. *Archives of Dermatology*, **76**, 437.

Holmes, E.J. (1968) Tumours of lower hair sheath. Common histogenesis of certain so-called 'sebaceous cysts' acanthomas and 'sebaceous carcinomas'. *Cancer*, **21**, 234.

Kligman, A.M. & Kirschbaum, J.D. (1964) Steatocystoma multiplex: a dermoid tumour. *Journal of Investigative, Dermatology*, **42**, 383.

Leppard, B.J. (1976) Epidermoid cysts. In preparation.

Leppard, B.J. & Bussey, H.J.R. (1975) Epidermoid cysts, polyposis coli and Gardner's syndrome. *British Journal of Surgery*, **62**, 387.

Leppard, B.J., Sanderson, K.V. & Wells, R.S. (1976) Hereditary trichilemmal cysts. Submitted for publication.

Lever, W.F. & Griesemer, R.D. (1949) Calcifying epithelioma of Malherbe: report of 15 cases, with comments on its differentiation from calcified epidermal cyst and on its histogenesis. *Archives of Dermatology and Syphilology*, **59**, 506.

Montagna, W., Chase, H.B. & Melaragno, H. (1952) The skin of hairless mice. I. The formation of cysts and the distribution of lipids. *Journal of Investigative Dermatology*, **19**, 83.

Muehlon, W.F. (1942) Zur Kenntnis des Malherbescen Epitheliom. *Scheizerische Zeitschrift für Pathologie und Bakteriologie*, **5**, 53.

Nicolaides, N., Levan, N.E. & Fu, H.C. (1968) The lipid pattern of the wen (keratinous cyst of the skin). *Journal of Investigative Dermatology*, **50**, 189.

Noble, W.C., Leppard, B.J. & Thomson, J. (1976) The bacteriology of skin cysts. In preparation.

Nürnberger, W. (1938) Die Epithelcysten der äusseren Haut und die aus ihnen neuergehenden Geschwülste. *Frankfurter Zeitschrift für Pathologie*, **52**, 448.

Peden, J.C. (1948) Carcinoma developing in sebaceous cysts. *Annals of Surgery*, **128**, 1136.

British Journal of Dermatology (1976) **94**, 391.

Wegener's granulomatosis

COMBINED THERAPY WITH LOW-DOSAGE SYSTEMIC CORTICOSTEROIDS, AZATHIOPRINE AND CYCLOPHOSPHAMIDE IN THREE PATIENTS

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SUMMARY

Three patients with Wegener's granulomatosis are reported. All three had skin, nose, sinus, mouth and pulmonary lesions; one had severe renal involvement as well. In two patients combined treatment with low-dosage systemic corticosteroids, azathioprine, and cyclophosphamide resulted in rapid clinical improvement and complete disappearance of the skin, mouth, nasal, sinus and pulmonary lesions. In one of these two patients who had severe renal involvement, proteinuria greatly diminished and arrest of progression of renal insufficiency was observed. In the third patient combined treatment with azathioprine and cyclophosphamide resulted in complete remission of skin, mucous membrane, lung and sinus lesions. The patient with severe renal involvement is alive and ambulant 4 years after the onset of the disease, the other two are completely symptom-free and well 2½ years and 1 year after the onset of their illnesses, respectively.

These results confirm previous reports about the effectiveness of immunosuppressive and cytotoxic agents in Wegener's granulomatosis, the use of which seems to have improved the prognosis significantly in a disease previously considered to be fatal within 5 months.

Wegener's granulomatosis is an uncommon condition believed to be an auto-aggressive disease probably related to periarteritis nodosa, allergic granulomatosis and lethal midline granuloma.

The first case was described by Klinger in 1931 but it was Wegener who describing three patients in 1936 and later in 1939 recognized this as a distinct clinical–pathological syndrome. Godman & Churg (1954) gave detailed pathological data of seven patients and set firm criteria for the diagnosis:

1. Necrotizing granulomatous lesions of the upper and lower respiratory tract.
2. Generalized necrotizing vasculitis involving both arteries and veins in many organs, of which the lungs are always involved.
3. Glomerulitis which may progress to granulomatous glomerulonephritis.

Carrington & Liebow (1966) described a limited form of the disease when the pulmonary lesions predominate and glomerulitis is absent, with a slightly better prognosis.

Walton (1958) in a retrospective study of his own ten patients and forty-six others selected from the literature concluded that about 83% of all patients die in renal failure and most of the others die of respiratory failure within 5 months, although a few lived up to 4 years.

Therapeutic efforts to modify the outcome of Wegener's granulomatosis, particularly with renal involvement, were disappointing (Walton, 1958), using radiotherapy (Merill, 1961), antibiotics and corticosteroids (Berman, Rydell & Eicheholtz, 1963; Fred *et al.*, 1964; Beidleman, 1963; Nielsen, Christiansen & Jensen, 1967).

In 1965, however, Greenspan reported success with the long-term administration of cyclophosphamide and prednisolone in three cases of lethal midline granuloma/Wegener's granulomatosis. In all cases skin, nose, mouth and pulmonary lesions regressed.

Bouroncle, Smith & Cuppage (1967) reported regression of nasal, pulmonary and renal lesions in a patient treated with azathioprine.

Norton, Suki & Strunk (1968), using combined corticosteroid and azathioprine treatment in two patients with Wegener's granulomatosis, achieved complete remission of all lesions lasting 17 months after beginning treatment.

Kaplan, Hayslett & Calabressi (1968) used azathioprine and duazomycin A (a glutamine antagonist) in two cases of Wegener's granulomatosis with remission of signs and symptoms of pulmonary and renal lesions.

Hutas, Miklos & Fenyes (1969), using azathioprine and corticosteroids in a case of Wegener's granulomatosis, reported complete remission of skin, nasal, pulmonary and renal lesions.

Raitt (1971), using a combination of corticosteroids and cytotoxic agents (either an alkylating agent or purine antagonist) in six cases of Wegener's granulomatosis, reported remission of all lesions and maintenance of renal function at a satisfactory or improved level. One patient died when bone marrow depression occurred after nitrogen mustard administration.

Kustner *et al.* (1971) reported three cases of Wegener's granulomatosis in which a combination of chlorambucil or azathioprine with low-dosage corticosteroids resulted in complete remission in all cases.

Capizzi & Bertino (1971) achieved complete remission in two cases of Wegener's granulomatosis with weekly intravenous injections of 50 mg methotrexate for 30 months.

Novack & Pearson (1971) treated four patients suffering from Wegener's granulomatosis with cyclophosphamide alone. In all four patients rapid amelioration of signs and symptoms occurred, lasting up to 3½ years.

Wishart (1975) reported a single case with pulmonary, renal and skin involvement in whom azathioprine and corticosteroids brought the disease under control. Pulmonary lesions recurred a fortnight after stopping the prednisolone. Restarting of systemic corticosteroids, however, brought back full control within a few weeks.

CASE REPORTS

Case 1

A 27-year-old man developed blockage of the nasal passages, headaches and had nasal polypectomy in 1964. His symptoms, however, persisted and in 1967 he was found to have infection of the ethmoid sinuses and had a left radical antrostomy and nasal polypectomy. He was not improving and in September 1970 he had a right-sided submucous resection of the septum and right sided nasal polypectomy. Three weeks later he developed a right middle ear effusion for which he had a right myringotomy. This resulted in some improvement, but in April 1971 he developed bilateral episcleritis and blood and albumin were found in his urine. A collagenosis was suspected and he was given a

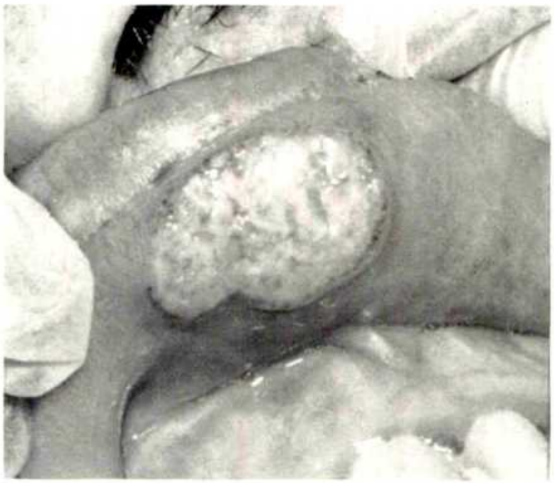

FIGURE 1. Case 1. Ulcerated lesion on inner aspect of upper lip and early lesion on right ala nasi.

4-weeks course of prednisolone with improvement of the episcleritis. In July 1971, tender nodules appeared on his fingers and lower legs. By December 1971, tender and painful nodules appeared in large numbers on his fingers, elbows, knees and lower legs with bleeding, ulcerating lesions appearing in his nose and mouth with marked halitosis and haemoptysis.

He was admitted to the Department of Dermatology at Hull Royal Infirmary at the beginning of January 1972, in a critically ill condition. His temperature was 39·4°C. He had marked halitosis; numerous punched-out, ulcerated lesions were present in the nasal passages, mouth (Fig. 1), throat, and on his fingers, elbows, knees and lower legs (Figs 2 and 3). There were several tender nodules on the palmar aspects of his fingers. There was dullness to percussion on the right side of his chest with absent pulmonary sounds. His ESR was 115 mm/h (Westergren); Hb 9·3 g/100 ml; WBC 18,000/mm^3, differential white count showed 46% eosinophils; blood urea was raised at 138 mg%; serum creatinine 1·5 mg/100 ml; 24 h urinary protein 23·5 g; faecal occult blood tests were repeatedly positive. Antinuclear antibodies were repeatedly negative; direct antiglobulin reactions were negative;

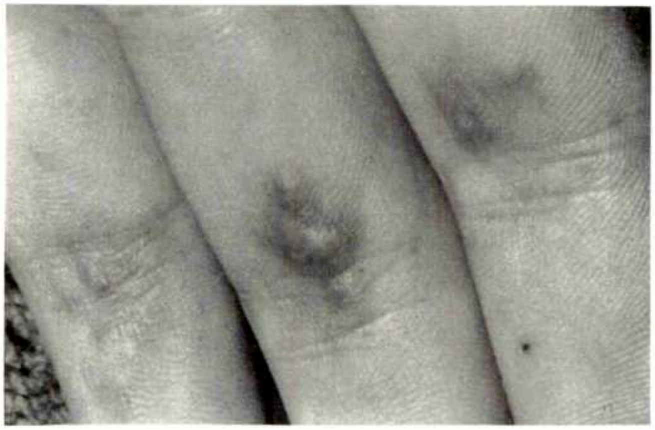

FIGURE 2. Case 1. Bluish nodular lesions on palmar aspects of fingers.

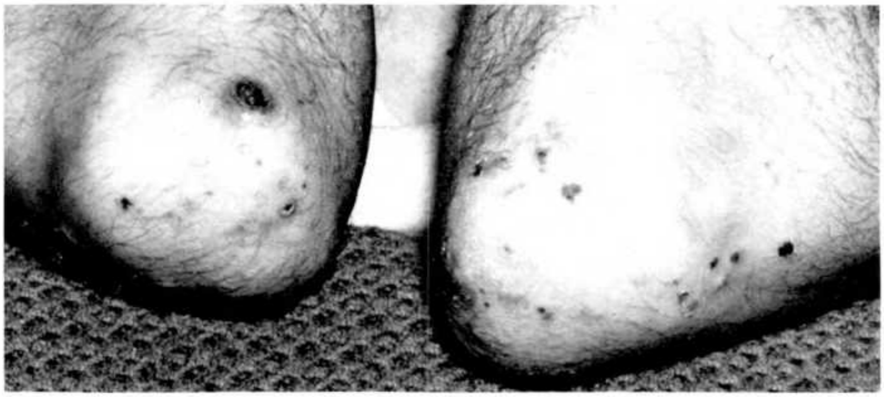

FIGURE 3. Case 1. Ulcerated and nodular lesions on elbows.

his urine contained blood and protein. A bone marrow biopsy showed a hyperplastic marrow with myeloid preponderance and eosinophilia. Skin and mucous membrane biopsies showed necrotizing granulomatous lesions in the dermis and small blood vessels, with fibrinoid necrosis and inflammatory cell infiltration of their walls (Figs 4 and 5). X-ray of chest showed shadowing of the right base with pleural effusion (Fig. 6).

A diagnosis of Wegener's granulomatosis was made. Treatment began with prednisolone 80 mg daily with improvement of skin and mucous membrane lesions but with little effect on proteinuria and oedema. By March 1972 he developed steroid-induced diabetes and by August 1972, hypertension and a duodenal ulcer. At this time prednisolone was reduced to 30 mg daily and azathioprine 50 mg

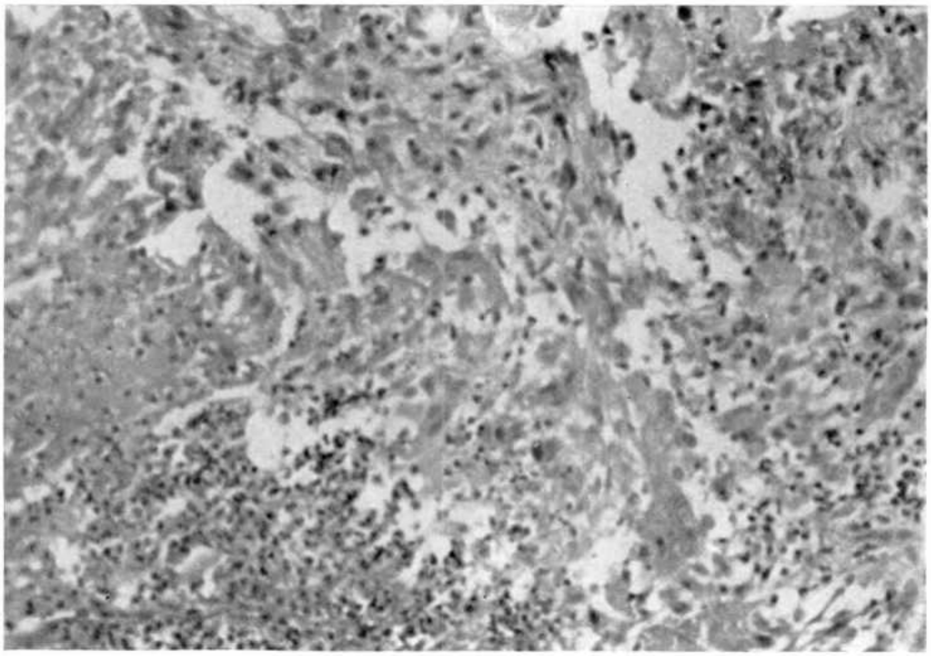

FIGURE 4. Case 1. Histology showing irregular necrosis in the dermis with granulomatous reaction.

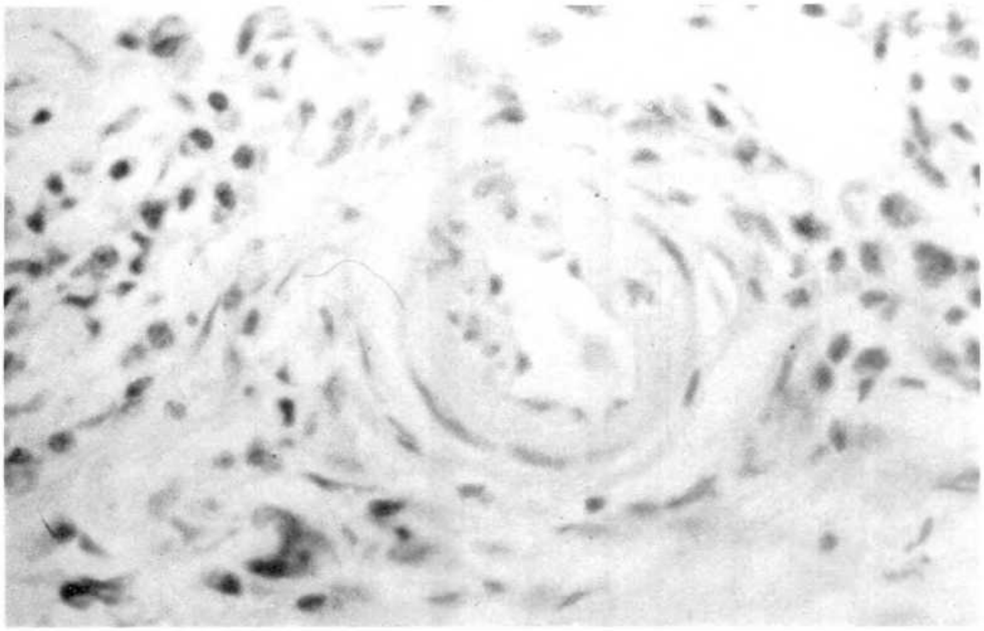

FIGURE 5. Case 1. Small blood vessel showing thickening of the endothelium and fibrinous deposits in vessel wall which is surrounded by a round cell infiltrate.

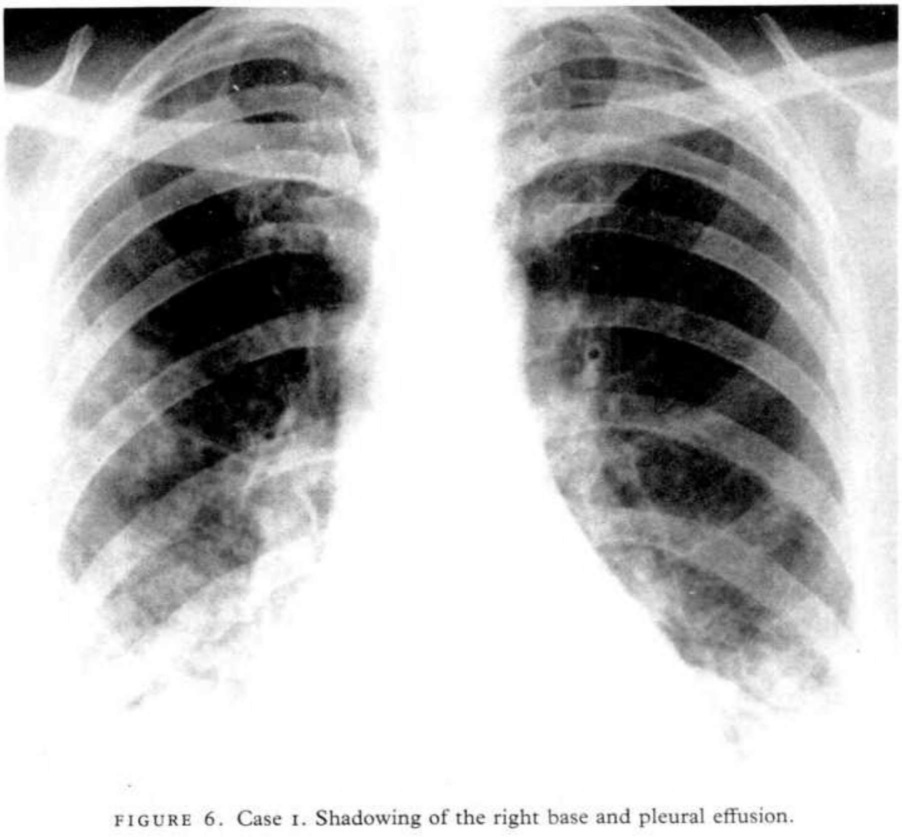

FIGURE 6. Case 1. Shadowing of the right base and pleural effusion.

twice daily was begun with a steady improvement of his general well being and reduction of proteinuria. In July 1973 prenisolone was reduced to 15 mg daily, azathioprine 50 mg twice daily was continued and he was started on cyclophosphamide 50 mg daily as well. This resulted in dramatic improvement. Urinary protein excretion fell to 1 g per 24 h. By November 1973 skin and mucous membrane lesions, diabetes and duodenal ulcer had cleared up and urinary protein output remained steady at 4–9 g per 24 h.

In April 1974 the patient contracted chickenpox from his son. He became toxic and was readmitted with a high temperature (around 40°C). A mixture of chickenpox and Wegener's granulomatosis lesions were present on the body and limbs. Hyperimmune gamma globulin 1·5 g was administered intramuscularly in a single dose. There was full recovery within 3 weeks.

At present he is on enteric-coated prednisolone 5 mg daily, azathioprine 50 mg twice daily and cyclophosphamide 50 mg daily. Skin, mucous membranes and lung lesions remain completely clear but albuminuria persists (2–7 g/24 h), with raised blood urea (around 50 mg/100 ml) and raised serum creatinine (around 2·2 mg/100 ml).

Case 2

This patient, a 60-year-old woman, developed a persistent purulent cough in the summer of 1973. In August 1973 she was admitted to hospital on account of severe dyspnoea, oedema of the legs and a persistent cough. There were coarse crepitations over both bases with diminished air entry in the left base. X-ray of chest showed bilateral inflammatory changes and left-sided pleural effusion. By September 1973 she complained of blocked nasal passages and was found to have bilateral sinusitis. Sinus lavages showed thick pus in both antra and a double intranasal antrostomy was carried out under the cover of several antibiotics.

In July 1973, she developed lumps on the lower legs which became progressively more tender and some of them became ulcerated. Later on, similar lesions appeared around the elbows and by December 1973 ulcers appeared in the nasal passages and mouth. In mid-January 1974 she was admitted to the Dermatological Ward in a critically ill condition. On admission she was greatly distressed by dyspnoea, haemoptysis, bilateral oedema of the feet and legs and pain and stiffness in the shoulders and back. Her temperature was elevated (37·8°C), there was dullness over both bases on percussion, and ulcerating, necrotic lesions were present in the mouth, nose and pharynx. There were tender, nodular and ulcerating lesions on both lower legs and around the elbows, with oedema of both feet and lower legs. Her ESR was 63 mm/h; Hb 10·8 g/100 ml; WBC 13,000/mm^3; antinuclear antibodies positive (titre 1:160); direct antiglobulin reaction negative; L.E. cell test negative; Rose Waaler test negative; serum creatinine 0·7 mg/100 ml; blood urea 27 mg/100 ml. Skin biopsies showed a granulomatous infiltrate in the dermis with the small dermal blood vessels showing endothelial swelling and proliferation and inflammatory cell infiltrate in their walls. X-ray of her chest showed extensive inflammatory changes in both bases and in the right mid-zone; 24 h urine contained 1·3 g of protein.

A diagnosis of Wegener's granulomatosis was made and she was put on azathioprine 50 mg twice daily and cyclophosphamide 50 mg twice daily simultaneously. On this regimen the mucous membrane and skin lesions cleared up completely within 6 weeks, dyspnoea improved and oedema of the legs and feet disappeared. Apart from a small area of inflammatory change in the right base that remained radiologically visible in April 1975, the chest lesions cleared up completely and haemoptysis disappeared. She remains mildly dyspnoeic. Azathioprine and cyclophosphamide were stopped in May 1974.

Case 3

This patient, a 44-year-old woman, following a summer holiday in 1973, developed a cough and noticed blood-stained sputum. Despite repeated courses of antibiotics she failed to improve and became dyspnoeic. Around Christmas 1973 she developed wheezing and was admitted to hospital. On auscultation coarse rhonchi were heard over the lungs; X-rays showed nonspecific inflammatory changes of both lungs and complete obliteration of the right maxillary sinus. The respiratory peak flow was reduced to 120 l/min. Her sputum was copious and purulent. On treatment with antibiotics the sputum cleared and dyspnoea improved. Respiratory peak flow went up to 280 l/min and she was discharged home. By Christmas 1974 her dyspnoea had increased greatly and by January 1975 she developed painful tender nodules on the backs of her hands and knuckles and over her knees; her left eye became bloodshot. She was admitted to the Department of Dermatology at the beginning of February 1975 in a fairly ill condition with raised temperature (around 37·8°C). She had bilateral episcleritis. Her tongue was ulcerated underneath and plaque-like ulcerated lesions were present over the knuckles, with very tender nodular lesions on the fingers, knees, toes, ankles and elbows. Coarse rales were heard over both lungs with expiratory wheezing.

Her ESR was 61 mm/h; Hb 10·6 g/100 ml; WBC 9,800/c mm (8% eosinophils); antinuclear antibodies positive (titre 1:160); direct antiglobulin reaction negative; immuno-electrophoresis IgG 220 i.u./ml; serum creatinine 0·2 mg/100 ml; 24 h urine 0·14 g; blood urea 2 mg/100 ml. X-ray of chest showed non-specific patchy inflammatory changes. X-ray of sinuses showed polypoidal mucosal swelling of the left antrum. Skin biopsies showed irregular necrosis in the dermis with a granulomatous reaction, the small blood vessels showing endothelial thickening and vessel walls infiltrated with inflammatory cells.

A diagnosis of Wegener's granulomatosis was made and treatment begun with prednisolone 15 mg daily, azathioprine 50 mg three times daily and cyclophosphamide 100 mg daily. Chest symptoms cleared up within 2 weeks, skin and mucous membrane lesions within 3 weeks, episcleritis after 6 weeks. She remains well and symptom-free on this treatment.

DISCUSSION

The cause of Wegener's granulomatosis remains obscure. Most authors, however, believe it belongs to the auto-aggressive diseases and is related to periarteritis nodosa, allergic granulomatosis and lethal midline granuloma (Rose & Spencer, 1956; Godman & Churg, 1954). Indirect supporting evidence for this classification would be the presence of antigen–antibody complexes in the glomeruli of diseased kidneys as shown by immunofluorescent techniques (Lerman & Castleman, 1969). More recently Burton & Burton (1972a, b) proposed a more dynamic concept of these conditions incorporating Löffler's syndrome to form the 'Chöffler Train'—a whole spectrum of syndromes characterized by varying degrees of pulmonary eosinophilia, necrotizing vasculitis and granulomatous proliferation, with fluidity of their boundaries.

Some authors (Tuhy, Maurice & Niles, 1958; Fahey *et al.*, 1954) maintain that histological changes in Wegener's granulomatosis suggest that it may be related to a hypersensitivity state. Germuth & Pollack's experiments (1967) gave a possible relationship of a hypersensitivity state to the granulomatous tissue changes. When rabbits were immunized repeatedly, the hyperimmune responders developed large amounts of insoluble antigen–antibody complexes in the circulation. The hyperimmune animals developed granulomatous lesions that were most prominent in the pulmonary veins and adjacent to pulmonary parenchyma.

This postulated relationship of Wegener's granulomatosis to hypersensitivity states made the therapeutic use of corticosteroids and immunosuppressive agents reasonable.

Walton in 1958 stated that most patients with Wegener's granulomatosis died within 5 months after the onset of their illness, only a few surviving up to 4 years. Radiotherapy (Merill, 1961) and corticosteroids (Berman *et al.*, 1963; Fred *et al.*, 1964; Beidleman, 1963; Nielsen *et al.*, 1967), only marginally improved the prognosis.

The introduction of: cyclophosphamide with corticosteroids (Greenspan, 1965); azathioprine (Bouroncle *et al.*, 1967); azathioprine and corticosteroids (Norton *et al.*, 1968); azathioprine and duazomycin-A (Kaplan *et al.*, 1968); the combination of corticosteroids and cytotoxic agents (Raitt, 1971); the combination of chlorambucil, azathioprine and low-dosage corticosteroids (Kustner *et al.*, 1971); methotrexate (Capizzi & Bertino, 1971); and cyclophosphamide (Novack & Pearson, 1971) seems to be a real advancement in the treatment of this potentially fatal condition.

Patients improved dramatically after the introduction of immunosuppressive and cytotoxic agents in all the reported cases. Lesions of the skin, mucous membranes and lungs were particularly rapid in their disappearance. Renal lesions were improved or arrested in all instances, even clearing up in a few cases. This is most gratifying as the cause of death was renal failure in about 83% of cases before immunosuppressive treatment was used. There is a suggestion also that early exhibition of these agents might avert renal involvement as seems up to now to be the case with two of the above reported cases. We are not in a position, as yet, to assess the long-term benefits of immunosuppressive treatment. Reports on this aspect of Wegener's granulomatosis are still awaited.

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REFERENCES

BEIDLEMAN, B. (1963) Wegener's granulomatosis. Prolonged therapy with large doses of steroids. *Journal of the American Medical Association*, **186**, 827.

BERMAN, D.A., RYDELL, R.E. & EICHEHOLTZ, A. (1963) Wegener's granulomatosis: A clinicopathologic study of four cases. *Annals of internal Medicine*, **59**, 521.

BOURONCLE, B.A., SMITH, E.J. & CUPPAGE, F.E. (1967) Treatment of Wegener's granulomatosis with Imuran. *American Journal of Medicine*, **42**, 314.

BURTON, J.L. & BURTON, PATRICIA A. (1972a) The Chöffler train. *Lancet*, **i**, 436.

BURTON, J.L. & BURTON, PATRICIA A. (1972b) Pulmonary eosinophilia associated with vasculitis and extravascular granulomata. *British Journal of Dermatology*, **87**, 412.

CAPIZZI, R.L. & BERTINO, J.R. (1971) Methotrexate therapy of Wegener's granulomatosis. *Annals of internal Medicine*, **74**, 74.

CARRINGTON, C.B. & LIEBOW, A.A. (1966) Limited forms of angiitis and granulomatosis of Wegener's type. *American Journal of Medicine*, **41**, 497.

FAHEY, J.L., LEONARD, E., CHURG, J. & GODMAN, G. (1954) Wegener's granulomatosis. *American Journal of Medicine*, **17**, 168.

FRED, H.L., LYNCH, E.C., GREENBERG, S.D. & GONZALES-AGULO, A. (1964) A patient with Wegener's granulomatosis exhibiting unusual clinical and morphological features. *American Journal of Medicine*, **37**, 311.

GERMUTH, F.G. & POLLACK, A.D. (1967) Immune complex disease. The granulomatous manifestations. *Johns Hopkins' Medical Journal*, **121**, 254.

GODMAN, G.C. & CHURG, J. (1954) Wegener's granulomatosis. Pathology and review of the literature. *Archives of Pathology (Chicago)*, **58**, 533.

GREENSPAN, E. (1965) Cyclophosphamide–prednisone therapy of lethal midline granuloma. *Journal of the American Medical Association*, **193**, 162.

HUTAS, I., MIKLOS, G.Y. & FENYES, D. (1969) Immunosuppressiv Therapia Wegener Granulomatosisban. *Orvosi Hetilap*, **110**, 2164.

KAPLAN, S.R., HAYSLETT, J.P. & CALABRESSI, P. (1968) Treatment of advanced Wegener's granulomatosis with Azathioprine and Duazomycin A. *New England Journal of Medicine*, **278**, 239.

KLINGER, H. (1931) Grenzformen der Periarteritis nodosa. *Franfurter Zeitschrift für Pathologie*, **42**, 455.

KUSTNER, W., LUBBERS, P., UTHGENANNT, H. & WEGENER, F. (1971) Die Wegenersche Granulomatose Diagnostische Probleme und klinischer Verlauf unter immunsuppressiver Therapie. *Schweizerische medizinische Wochenschrift*, **101**, 1137.

LERMAN, J. & CASTLEMAN, B. (1969) Draining ears and renal failure in a 36-year-old man. *New England Journal of Medicine*, **280**, 828.

MERILL, M.D. (1961) Roentgen therapy in Wegener's granulomatosis. *American Journal of Roentgenology, Radium Therapy and Nuclear Medicine*, **85**, 96.

NIELSEN, K., CHRISTIANSEN, I.B. & JENSEN, E. (1967) Wegener's granulomatosis. A survey of three cases. *Acta medica Scandinavica*, **181**, 577.

NORTON, W.L., SUKI, W. & STRUNK, S. (1968) Combined cortico-steroid and Azathioprine therapy in two patients with Wegener's granulomatosis. *Archives of internal Medicine*, **121**, 554.

NOVACK, S.N. & PEARSON, C.M. (1971) Cyclophosphamide therapy in Wegener's granulomatosis. *New England Journal of Medicine*, **284**, 938.

RAITT, J. (1971) Wegener's granulomatosis: Treatment with cytotoxic agents and adrenocorticoids. *Annals of internal Medicine*, **74**, 344.

ROSE, G.A. & SPENCER, H. (1956) Periarteritis Nodosa. *Quarterly Journal of Medicine*, **XXVI** (new series), 43.

TUHY, J.E., MAURICE, G.L. & NILES, N.R. (1958) Wegener's granulomatosis. *American Journal of Medicine*, **25**, 638.

WALTON, E.W. (1958) Giant-cell granuloma of the respiratory tract (Wegener's granulomatosis). *British medical Journal*, **ii**, 265.

WEGENER, F. (1936) Über generalisierte, septische gefässerkrankungen. *Verhandlungen der Deutschen Pathologischen Gesellschaft*, **29**, 202.

WEGENER, F. (1939) Über eine eigenartige rhinogene Granulomatose mit besonderer Beteiligung des Arteriensystems und der Nieren. *Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie*, **102**, 36.

WISHART, J.M. (1975) Wegener's granulomatosis—controlled by azathioprine and corticosteroids. *British Journal of Dermatology*, **92**, 461.

British Journal of Dermatology (1976) **94**, 401.

Challenge test battery in chronic urticaria

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SUMMARY

The use of a battery of challenge tests in the routine investigation of patients with chronic urticaria has an important place, demonstrating an exacerbating factor in over half the patients. Diets designed to reduce the ingestion of the substances concerned are associated with clearing or considerable improvement in 75% of the patients treated.

A number of substances, many of which are present in foods and drugs, may have an exacerbating effect in some patients with chronic urticaria. This has been shown by a number of workers, notably Juhlin, Michaëlsson, & Zetterstrom (1972), Michaëlsson & Juhlin (1973), and more recently Doeglas (1975). These include the following:

1. Salicylates chiefly taken in drug form, but also to some extent occurring naturally in foods.
2. Other drugs including particularly indomethacin.
3. Tartrazine and other azo dyes occurring in many coloured foods and drugs, including many coloured antihistamine tablets.
4. Sodium benzoate and 4-hydroxybenzoic acid used as preservatives in foods, such as pickles, sauces, fruit juices, instant coffee, preserves, artificial cream and some tinned foods.
5. Brewer's yeast and Candida in the gut, possibly acting through a specific immunological mechanism, but there is considerable evidence of a more general exacerbating effect.

Penicillin causes urticaria usually through an allergic mechanism, but in some cases it may have a general exacerbating action (Warin & Champion, 1974).

In an individual patient with chronic urticaria, the only practical way to determine whether or not any of the substances have such an exacerbating effect is to administer test doses. The object of this present work was, by altering doses, timings and substances and by interspersing controls, to develop a test battery which can be administered in a routine way as part of the investigation of a patient with chronic urticaria.

METHOD

The 111 patients so far tested had all had ordinary urticaria for at least 2 months. The average age was 39 years, and the range 3–79 years. In this particular study we excluded dermographism, cholinergic urticaria and the other physical urticarias. Five patients were tested after admission to hospital, but the remainder were out-patients. A normal diet was continued throughout the test period.

Antihistamine therapy was continued, if from the aspect of clinical management this was necessary, but the dose was reduced as far as possible, so that the patient had some relief; the weals in most cases were still developing. This same dose was then continued throughout the whole test period. Antihistamines were not stopped for two reasons. First, in many cases this would lead to an exacerbation during the test, which may take a few weeks, and patients might find this difficult to bear. Secondly, it makes interpretation of minor positive reactions more difficult. Moderate reactions will nearly always break through the suppressive effect of antihistamines. This was known from previous work with aspirin. It is realized that we may well have missed some minor reactions, but the aim has been to establish a challenge test battery which can be used as a routine investigation in out-patients.

The test substances were incorporated into identical and unmarked capsules, and were administered one every morning. Lactose was used as a filling, and for the control capsules. The patients had a chart, and each day made a note of the severity of the weals on that day, the times when exacerbations occurred, and if any obvious cause was apparent to the patient. After the test period the chart was examined, and if there were any exacerbations recorded, the substances apparently responsible, together with suitable controls were then re-administered, in an altered order. A second chart was filled in, and if in this second test period there was again an exacerbation with a particular substance, this was recorded as giving a positive result.

TABLE 1. Pilot battery

A. Control Candida (liquid)
B. Candida (liquid)
1. Control
2. Tartrazine 1 mg with lactose
3. Tartrazine 10 mg with lactose
4. Control
5. Sodium benzoate 50 mg
6. Sodium benzoate 500 mg
7. 4-Hydroxybenzoic acid 50 mg
8. 4-Hydroxybenzoic acid 200 mg
9. Control
10. Brewer's yeast extract 0·4 g
11. Tartrazine 10 mg with kaolin
12. Tyramine 100 mg
13. Penicillin G 1 mg
14. Penicillin G 125 mg
15. Aspirin 150 mg
16. Aspirin 600 mg

Tests 3, 6, 8, 14 and 16 not to be taken if the weals increase with the previous test dose.

Various chemicals, doses, and timings have been tested, and the first battery elaborated is shown in Table 1.

After investigating the first forty patients with this Pilot Battery, various matters became apparent. *Candida albicans* extract reactions closely paralleled yeast reactions, and as yeast is so much easier to obtain, it seemed unnecessary to add Candida extract which was the only substance which had to be given in liquid form.

In some preliminary work the filler used for the capsules was kaolin. There was no reaction at all to tartrazine in these cases, and as Michaëlsson and Juhlin had used lactose it was considered that kaolin might in some way be binding the tartrazine. Later when reactions occurred to tartrazine with lactose they usually occurred to tartrazine with kaolin.

Reactions with tartrazine sodium benzoate and 4-hydroxybenzoic acid usually occurred with the bigger dose and were often equivocal with the smaller. No severe reactions occurred but occasional cases have been reported when these have developed in response to doses in the range of the smaller amount.

Tyramine evoked only occasional and somewhat doubtful reactions. A yeast-low diet and tyramine-low diet as used in migraine are very similar, and it had been thought that yeasts and Candida might act by liberating tyramine or some other substance in the gut, which might then have an exacerbating effect.

Penicillin 125 mg equivalent to 200,000 units is perhaps too risky, as some of these patients might show an extreme allergic response.

TABLE 2. Second battery

1. Control
2a. Tartrazine 1 mg with lactose (instead of 2 if history of asthma)
2. Tartrazine 10 mg with lactose
3. Control
4a. Sodium benzoate 50 mg (instead of 4 in children under 10 years)
4. Sodium benzoate 500 mg
5. Control
6a. 4-Hydroxybenzoic acid 50 mg (instead of 6 in children under 10 years)
6. 4-Hydroxybenzoic acid 200 mg
7. Control
8. Brewer's yeast extract 0·6 g
9. Control
10. Penicillin G 0·5 mg (not to be given if history of severe penicillin reactions)
11. Control
12. Aspirin 100 mg
13. Aspirin 600 mg (not to be given if history of asthma or severe aspirin reactions)

A severe reaction evoked by the test substance may take a few days to settle down. This is particularly so with aspirin, which was therefore administered last in the series of tests. It would be preferable to allow a longer time between the test doses and it might be better to give the capsules on alternate days. However, this would mean that the whole test would take 5–6 weeks.

After the first forty patients, the battery was therefore changed to that given in Table 2.

Again the capsules were administered every day, but a control was given between each substance. This battery can be completed in 2 weeks.

The smaller dose of tartrazine was given if there was a history of asthma. The larger doses of sodium benzoate and 4-hydroxybenzoic acid were given, except in children under 10 years old. The penicillin dose was reduced to 0·5 mg (800 units) and not given if there was a history of severe penicillin reactions. The larger dose of aspirin was not given if there was a history of asthma or severe aspirin reactions.

TABLE 3

Exacerbations	No.	% of total no. of patients
Aspirin	45	41
Sodium benzoate	12	11
4-Hydroxybenzoic acid	5	5
Tartrazine	14	13
Brewer's yeast and Candida	15	14
Penicillin	17	15
Total	108	

Total number of patients 111.
Exacerbations 66. No exacerbations 45.

RESULTS

The results in the 111 patients, namely forty tested with the pilot battery and the remainder with the second battery, are summarized in Table 3.

COMMENTS

The number of aspirin reactors is 41% as compared with figures previously reported, ranging from 20 to 40% (Warin, 1960; James & Warin, 1970). The number of tartrazine reactors, 13%, is much lower than reported in the work of Juhlin *et al.* (1972) who gave an incidence of over 50% reacting to azo dyes, and Doeglas (1975) who reported 30% reacting to tartrazine. However, all his subjects tested were known aspirin reactors. Of our aspirin reactors, 25% also had tartrazine reactions. The number reacting to benzoic acid derivatives is also far below Michaëlsson and Juhlin's figure of over 50%. A possible explanation for this may be that Michaëlsson and Juhlin stopped all antihistamine treatment, and so in their patients minor reactions might have developed.

Brewer's yeast reactors at 14% are also well below the figure of 24% obtained in previous work (James & Warin, 1971). The penicillin results are interesting; five of the seventeen patients were known to have had urticaria from penicillin in the past.

It will be noticed from the numbers of reactions recorded that some patients showed an exacerbation with two or more substances. This occurred particularly with aspirin and penicillin (twelve patients), with aspirin and tartrazine (eleven patients), and with aspirin and sodium benzoate (eight patients).

In most of the previous reports very small doses of the test substances were given initially and the amount then increased every 2 h. This method might be applicable to patients who have been admitted for the tests but would be difficult to manage in out-patients. Also, as the reaction may occur as long as 8–24 h after the dose, there could be more danger of severe reactions developing.

As the patients were on normal diets there may well have been some exacerbations from the coincidental ingestion of the substances concerned. It might well be preferable to put patients on a diet with a low content of yeasts, benzoic acid preparations and azo dyes throughout the test period.

The course of chronic urticaria fluctuates and exacerbations occur for no apparent reason. It was therefore to be expected that these developed at times after the placebo tablet had been given, and therefore may have also coincided with the test doses and so given erroneous results. It was therefore important to repeat the test doses, again interspersed with controls and so reduce the likelihood of false positive reactions.

It is likely that the challenge test battery will need to be altered in the future, and other substances may well need to be added, but in its present form and at our present state of knowledge it can be used satisfactorily in a routine way in out-patients.

Diet treatment

Diet sheets have been prepared, which reduce, as far as possible, the content of salicylates, benzoic acid preparations, azo dyes, and yeasts. These have been modified and simplified from diets prepared by Juhlin *et al.* (1972), Cunliffe (1975) and Holti (1966). They will obviously need modification in the future and according to national diet habits. The patients who gave a positive reaction to one or more substances were given the appropriate diet and the results were assessed after 2 months. Work with these diet treatments is not completed, but up to the present time it is possible to assess results in forty-seven patients. Thirty-five (75%) either cleared or improved considerably, and in three there was no effect. Five were impossible to assess, usually because the urticaria was only slight before the diet was started, and four failed to keep to the diets satisfactorily. This 75% improvement is in line with the results obtained by others, including Michaëlsson & Juhlin (1973) and Doeglas (1975). If improvement had occurred, the diet was continued and the antihistamine, if still taken, was gradually stopped. The patients were instructed gradually to relax the diet regime over the course of the next few months.

Many of the thirty-five patients cured or improved were able to revert to normal diets after 5–6 months. This fact is not evidence against the substances concerned being causal factors in the urticaria, as it has been shown with aspirin reactors that when the phase of urticaria has cleared they can often take aspirin without getting weals (Moore-Robinson & Warin, 1967).

It must, however, be pointed out that many patients with chronic urticaria improve spontaneously after a period of some 6–12 months, and by the time these patients have waited for referral, had the investigations, and been on the diet for 2 months, many would have cleared anyway. Furthermore, it has been shown that 'suggestion' may play an important part in the improvement or cure of chronic urticaria, and this must be allowed for in considering results.

Assessment of the effect of these diet treatments is still continuing, but they are apparently well worthwhile.

ACKNOWLEDGMENT

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REFERENCES

Cunliffe, W.J. (1975) Personal communication.

Doeglas, H.M.G. (1975) Reactions to aspirin and food additives in patients with chronic urticaria, including the physical urticarias. *British Journal of Dermatology*, **93**, 135.

Holti, G. (1966) Candida allergy. *Symposium on Candida Infections* (Ed. by H.L.Winner and R.Hurley), p. 73. Livingstone, Edinburgh.

James, J. & Warin, R.P. (1970) Chronic urticaria: the effect of aspirin. *British Journal of Dermatology*, **82**, 204.

James, J. & Warin, R.P. (1971) An assessment of the role of Candida albicans and food yeasts in chronic urticaria. *British Journal of Dermatology*, **84**, 227.

Juhlin, L., Michaëlsson, G. & Zetterstrom, O. (1972) Urticaria and asthma induced by food and drug additives in patients with aspirin hypersensitivity. *Journal of Allergy and Clinical Immunology*, **50**, 92.

MICHAËLSSON, G. & JUHLIN, L. (1973) Urticaria induced by preservatives and dye additives in food and drugs. *British Journal of Dermatology*, **88**, 525.
MOORE-ROBINSON, M. & WARIN, R.P. (1967) Effect of salicylates in urticaria. *British Medical Journal*, **iv**, 262.
WARIN, R.P. (1960) The effect of aspirin in chronic urticaria. *British Journal of Dermatology*, **72**, 350.
WARIN, R.P. & CHAMPION, R.H. (1974) *Urticaria*. W.B.Saunders, Philadelphia.

British Journal of Dermatology (1976) 94, 407.

Histological aspects of DNCB sensitization and challenge tests

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SUMMARY

I. A group of twenty-one Caucasian volunteers was patch tested without preliminary sensitization with 3, 10, 30, 50 and 90 μg 2,4-dinitrochlorobenzene (DNCB)/cm^2. Of this group seven persons were read after 24 h; with one exception all showed a toxic erythematous reaction to 30 μg and higher amounts of DNCB/cm^2. The other fourteen volunteers were read after 48 h. All except one reacted positively to 30 μg and higher amounts of DNCB/cm^2. Histologically no changes could be noted after application of 3 and 10 μg DNCB/cm^2; they started to appear in patch tests with 30 μg DNCB/cm^2 or more.

II. A second group of twenty-one Caucasian volunteers was sensitized with 2000 μg DNCB on a surface area of 3·14 cm^2. Biopsies were performed after 1 h, 5 h, 1 day and then daily for 17 days. The histological changes occurring in the sensitization site during the primary irritant reaction of the first days were compared with the changes occurring during the flare-up reaction. No essential qualitative differences could be noted.

III. Four Caucasian volunteers were sensitized with 2000 μg DNCB on 3·14 cm^2. Fourteen days later challenge patch test doses of 3, 10, 30, 50 and 90 μg DNCB/cm^2 were applied. Biopsies of the challenge sites were performed after 48 h. In contrast to the results in group I, histological changes could already be noted after challenge patch tests with 3 μg DNCB/cm^2. The nature of the histological changes in primary irritant and allergic reactions to DNCB appeared to be identical and consisted of spongiosis, epidermal degeneration and lymphocytic infiltration around vessels and epidermal appendages and penetrating into the epidermal layers.

It is concluded that, in order to avoid errors through misinterpretation, challenge patch tests with DNCB must be performed with low amounts, e.g. 3 or 10 μg/cm^2.

Sensitization and challenge tests with 2,4-dinitrochlorobenzene (DNCB) are increasingly used as one of the parameters of cellular reactivity. Sensitization to DNCB is commonly induced with an amount of 2000 μg DNCB applied within an area of 2 cm diameter (surface area 3·14 cm^2). A survey of the different amounts of DNCB applied per cm^2 skin surface in challenge tests, as mentioned by different authors, is given in Table 1.

Clinically it is often difficult to decide whether skin reactions to externally applied compounds are

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TABLE 1. Amounts of DNCB used for challenge tests by different authors

Author	μg DNCB/cm²	Test method
Haxthausen (1939)	500	Open
Rostenberg & Kanof (1941)	0·7	Open
Gross (1965)	26	Closed
Walker (1967)	4	Open
Turk & Waters (1969)	8	Open
	16	
Eilber & Morton (1970)	13	Closed
	26	
Catalona *et al.* (1972)	16	Open
Bleumink *et al.* (1974)	3	Closed
	10	
	30	

caused by primary irritant effects or by contact sensitization. This difficulty is especially of importance in patients where DNCB sensitization and challenge tests are performed, as DNCB, apart from being a sensitizer, also causes strong primary irritant effects. A previous study revealed that patch tests with 80 μg DNCB/cm^2 skin in persons not previously sensitized led after 48 h to a primary irritant reaction in sixteen of eighteen normal control persons (Bleumink *et al.*, 1974).

Clinically the differentiation between these toxic skin reactions and an allergic patch test response may cause difficulties and lead to an erroneous interpretation. Several authors (e.g. Wilhelm, Sarkany & Calnan, 1958; Bandmann, 1960) have tried to differentiate between toxic and allergic responses by means of a histological examination of the patch-test reaction. Based on the literature and on their own investigation Wilhelm *et al.* (1958) mentioned the following similarities and differences:

(a) In toxic reactions there is more tendency towards partial detachment of the horny layer from the rest of the epidermis. In some sections only a small accumulation of homogeneous eosinophilic staining material may be noted.

(b) The epidermis in toxic reactions shows a variable degree of necrotic changes leading to an alteration of the staining properties.

(c) Spongiosis and vesicle formation are similar to those found in the allergic group. They do not necessarily imply an allergic origin.

(d) Changes in the cutis are similar to those in the allergic group: perivascular round cell infiltration occurs, most markedly in the upper parts of the cutis. The infiltration round the appendages is somewhat less marked in the toxic group. Bandmann (1960) gives the following differential diagnostic criteria:

(a) In the early stages of an allergic reaction a basal focal spongiosis with lymphocytic infiltration occurs. Somewhat later a lymphocytic perivascular infiltration of the upper corium may be noted.

(b) In a later phase of an allergic reaction an almost pure lymphocytic perivascular infiltration of the corium occurs. In toxic reactions this infiltration develops in a markedly weaker degree and is according to the author of a more mixed histiocyte-lymphocyte composition.

Lachapelle (1972) proposed ^{3}H-thymidine labelling as an additional tool to distinguish between allergic and toxic reactions.

The investigations of Wilhelm *et al.* (1958) as well as of Bandmann (1960) were performed with different compounds causing either toxic or allergic reactions. The possibility must not be excluded that part of the differences noted were caused by the different chemical structures of the compounds. The purpose of the present investigation was to examine the histological reaction following:

I. The application of DNCB in amounts which are commonly advocated for challenge tests: 3, 10, 30, 50 and 90 μg/cm^2 without preliminary sensitization. This part of the study was carried out to ascertain which amount of DNCB/cm^2 applied on an unsensitized skin does not give rise to clinically visible toxic reactions and/or to histopathological changes.

II. The application of a sensitizing dose of 2000 μg DNCB.

III. The application of DNCB in challenge doses of 3, 10, 30, 50 and 90 μg/cm^2 after preliminary sensitization with 2000 μg DNCB.

MATERIALS AND METHODS

I. The first group consisted of twenty healthy Caucasian volunteers, without any previous history of eczema, and one patient with an extensive contact eczema of the hands and the arms, who was included in this group by accident. The age of the individuals varied from 20 to 49 years. There were 7 females and 14 males. All were, without preliminary sensitization, patch tested on the skin of the back with 3, 10, 30, 50 and 90 μg DNCB dissolved in 0·01 ml acetone, applied on patch testers. The surface of the square Whatman paper, on which the dose was applied, was 1 cm^2.

The group was divided in the following way:

(a) In seven persons the tests were read after 24 h. From two persons a total of five biopsies were taken. In addition from both a control biopsy was taken of normal skin from the opposite side of the back.

(b) The other fourteen volunteers were read after 48 h. From six persons a total of fourteen biopsies was performed. Furthermore, from every biopsied person a control biopsy was taken of normal skin from the opposite side of the back.

All biopsies taken are indicated by an asterisk in Table 2.

II. The second group consisted of twenty-one normal healthy Caucasian volunteers without a prior history of skin diseases. The group included sixteen interns aged 24–37 years and five persons aged 41, 48, 51, 73 and 82 years respectively. The total group consisted of 1 female and 20 males. All subjects were sensitized with 2000 μg DNCB (British Drug House and recrystallized twice in order to remove impurities) which was applied topically, 0·1 ml of a 2% solution in acetone, within an area of a polyethylene ring, 2 cm diameter (i.e. 637 μg DNCB/cm^2) on the volar surface of the right arm.

Two weeks after sensitization the extension of the flare-up or remaining erythema reaction was measured. Furthermore challenge tests were performed with 3, 10 and 30 μg DNCB dissolved in 0·01 ml acetone applied on patch tests with a surface of 1 cm^2. The tests were performed on the skin of the back and read after 48 h. Punch biopsies of the sensitization sites were performed in the following way: two volunteers were biopsied after 1 and 5 h, two others after 24 and 48 h, two after 3 and 4 days and so on up to 17 days. In all persons at the same time a control biopsy was taken from a controlateral untreated area on the left arm.

III. Four normal healthy male Caucasian volunteers aged 27, 32, 54 and 60 years respectively without a prior history of skin diseases were sensitized to DNCB according to the method used in group II. Two weeks after sensitization, challenge patch tests were performed on the skin of the back. Patch tests with 3, 10, 30, 50 and 90 μg DNCB/cm^2 dissolved in 0·01 ml acetone were applied. After 48 h, 3 mm punch biopsies were taken from the sites on which the challenge dose had been applied.

All patch-test reactions were read according to the rules laid down by the International Contact Dermatitis Research Group (Bandmann & Fregert, 1973). The biopsies were performed after local

TABLE 2. Results of patch tests with different amounts of DNCB on twenty-one non-sensitized persons (group I)

Patient no.	Age	Sex	3 μg	10 μg	30 μg†	50 μg	90 μg	Control biopsy normal skin
A. Reading after 24 h								
1	25	M	—	—	e	e	e	*
2	24	F	—	—*	e*	e*	e	*
3	21	F	—	—	e*	e	e*	
4	24	F	—	—	e	e	e	
5	21	M	—	—	—	e	e	
6	27	M	—	—	e	e	e	
7	29	M	—	—	e	e	e	
B. Reading after 48 h								
8	49	M	—*	—	e*	e	e*	*
9	37	M	e	e	e	e	e*	*
10	39	M	—	—	e	e	e	
11	22	F	—	—	e*	e	e	*
12	25	M	—	—	e*	e*	e*	*
13	23	M	—	—*	e*	e*	e	*
14	23	M	—*	—*	e*	e	e	*
15	43	M	—	—	e	e	e	
16	22	F	—	—	e	e	e	
17	24	M	—	—	e	e	e	
18	29	F	—	—	e	e	e	
19	33	F	—	—	e	e	e	
20	19	M	—	—	e	e	e	
21	26	M	—	—	e	e	e	

* Biopsy. † e = Erythema.

chlorethyl anaesthesia with 3 mm punches. The biopsy specimens were fixed in neutral formalin, dehydrated and embedded in paraffin. Sections of 4 μg were stained routinely with haematoxylin-eosin and Giemsa.

RESULTS

1. The first group of twenty-one volunteers was patch tested without preliminary sensitization with 3, 10, 30, 50 and 90 μg DNCB/cm^2. Of this group seven persons were read after 24 h. The results (see Table 2) indicate that with only one exception all showed a toxic erythematous sharply defined reaction, when tested with 30 μg or higher amounts of DNCB.

The other fourteen volunteers of this group, who were read after 48 h, showed an identical erythematous sharply defined and morphologically toxic reaction to 30 μg and higher amounts of DNCB/cm^2. The one exception was a man (No. 9), who in contrast to the rest of the group at the time of patch testing was under treatment for an extensive allergic contact dermatitis caused by epoxy resin. He was the only non-healthy person in the group. This man reacted with an erythematous reaction to 3 μg, 10 μg and higher amounts of DNCB.

Histology after application of DNCB without prior sensitization: As is shown in Fig. 1 the amount of 3 or 10 μg DNCB/cm^2 applied on the skin does not cause any histological changes in the surface epithelium or in the dermis.

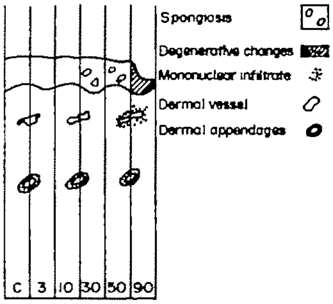


FIGURE 1. Schematic drawing of changes after application of 3, 10, 30, 50 and 90 μg of DNCB without prior sensitization, in comparison to the control skin of the same patient (C).

Surface epithelium changes start to appear in the form of spongiosis after application of 30 μg and increase to degenerative changes after application of 90 μg. At 50 and 90 μg perivascular dermal infiltrate can also be observed.

The lesions start to appear after application of 30 μg DNCB. In these cases a slight spongiosis is seen in the surface epithelium. This spongiosis increases after application of 50 μg and with this amount a little perivascular infiltrate in the dermis is also seen. After the application of 90 μg DNCB a perivascular infiltrate as well as degenerative changes of the surface epithelium can be observed.

II. The second group of twenty-one volunteers was sensitized with 2000 μg DNCB on the forearm. Fourteen days later the extension of the flare-up or remaining erythema reaction was measured and challenge patch tests were performed on the back with 3, 10 and 30 μg DNCB. The results (see Table 3) indicate positive challenge patch test reactions to all amounts of DNCB in all but three persons. The flare-up reaction was scored as positive in fifteen cases, negative in three and indefinite in three cases.

Histology after application of a DNCB sensitization dose of 2000 μg on the volar side of the right underarm (see Fig. 2).

1–5 h. In comparison to a control biopsy from an untreated area on the left underarm (Fig. 3) the histology does not reveal any abnormalities; only a slight mononuclear perivascular infiltrate is seen in the upper layers of the dermis (Fig. 4).

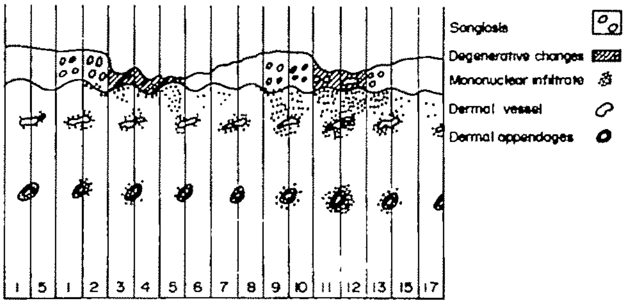


FIGURE 2. Schematic drawing of dermal changes after application of 2 mg DNCB after varying periods. In the first two columns the numbers (1, 5) represent hours, in the rest of the columns the numbers represent days. It can be observed that the dermal changes are biphasic in character. The first changes start to appear after a few hours and become more severe from day 1 until day 5, in which spongiosis, degenerative changes of the surface epithelium and dermal infiltrate appear. Then after day 6 these changes subside, to reappear from day 9 to day 13. Although principally there is no difference from the changes occurring from day 1 to day 5, the dermal infiltrate is more severe and the degenerative changes of the surface epithelium are somewhat less pronounced.

TABLE 3. Results of DNCB challenge patch tests in twenty-one sensitized persons

Patient no.	Age	Sex	Flare-up after 14 days	Extension of flare-up or remaining erythema (mm)	Challenge test reactions		
					3 μg	10 μg	30 μg
1	32	M	+	40 × 37	1	2	2
2	28	M	+	45 × 48	4	4	4
3	27	M	+	47 × 43	4	4	4
4	34	M	+	42 × 40	3	4	4
5	26	M	+	69 × 54	4	4	4
6	27	M	+	37 × 37	3	3	4
7	73	M	−	22 × 21	0	1	1
8	82	M	−	21 × 20	0	0	1
9	27	M	+	56 × 34	4	4	4
10	24	M	±	41 × 38	2	3	3
11	24	M	+	39 × 40	3	4	4
12	26	M	−	30 × 26	2	2	3
13	23	M	+	40 × 58	4	4	4
14	25	M	±	39 × 40	3	4	4
15	25	M	+	42 × 45	4	4	4
16	31	M	+	60 × 39	4	4	4
17	41	M	+	45 × 48	4	4	4
18	51	M	+	44 × 46	2	3	3
19	48	F	±	45 × 45	2	2	3
20	28	M	+	60 × 54	3	3	4
21	28	M	+	53 × 44	3	3	3

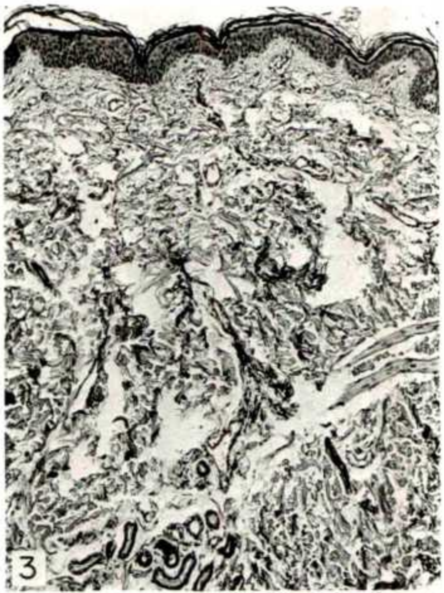
1–2 *days*. The mononuclear perivascular infiltrate increases and also some mononuclear infiltrate can be seen around the dermal appendages. The epithelium shows some spongiosis which increases with time. At the end of day 2 some mononuclear infiltrate is seen also round the basal layer of the epidermis.

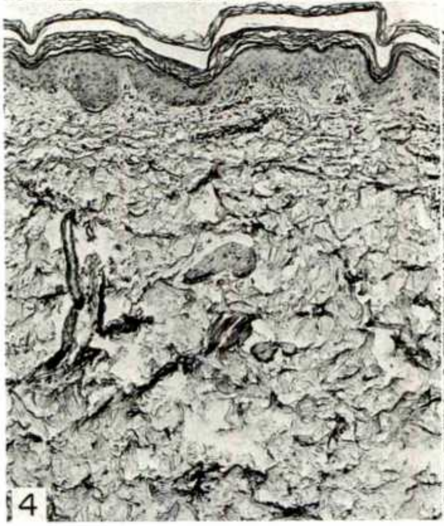
Day 3–*day* 5. The skin shows marked abnormalities. There is an increased mononuclear infiltrate around the dermal vessels, around the basal layer of the epithelium and around the dermal appendages. The epithelium undergoes necrotic changes and in most of the cases is shed (Fig. 5).

Day 6–*day* 8. The changes gradually diminish. The perivascular infiltrate diminishes and the infiltrate around the dermal appendages disappears. There is a marked regeneration of the surface epithelium which by the end of day 8 is normal again.

Day 9–*day* 10. The perivascular infiltrate in the upper layers of the dermis increases again. Also mononuclear cells around the dermal appendages are seen. The surface epithelium again shows spongiotic changes.

Day 11–*day* 12. The perivascular infiltrate and the infiltrate around the dermal appendages increases considerably, the surface epithelium shows degenerative changes and increased spongiosis (Fig. 6). In a few cases this surface epithelium is shed, but in most cases the changes are not as marked as those seen from day 3 to day 5.





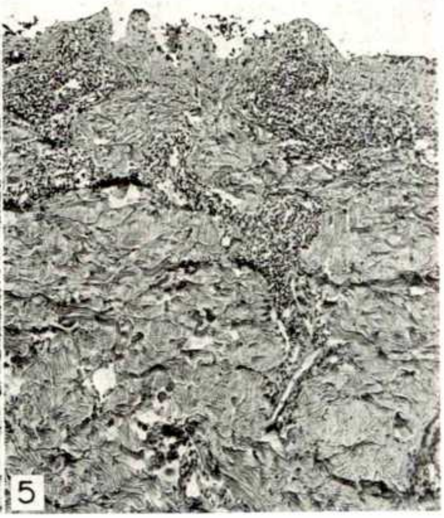


FIGURE 3. Normal skin, used as a control corresponding with 'C' in Figs 1 and 3 (H & E, × 80).

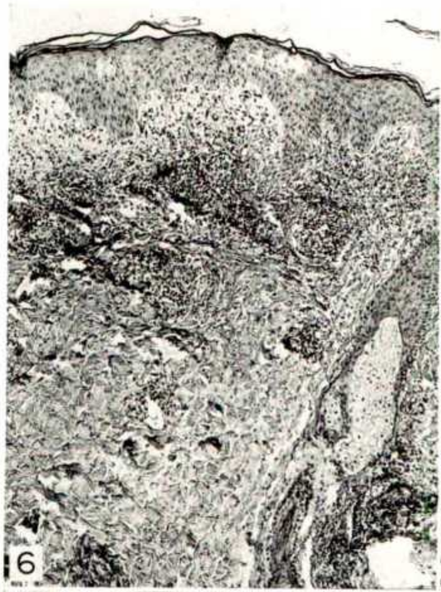
FIGURE 4. Dermal changes 5 h after application of 2 mg of DNCB, corresponding with the second column of Fig. 2 (H & E, × 80)

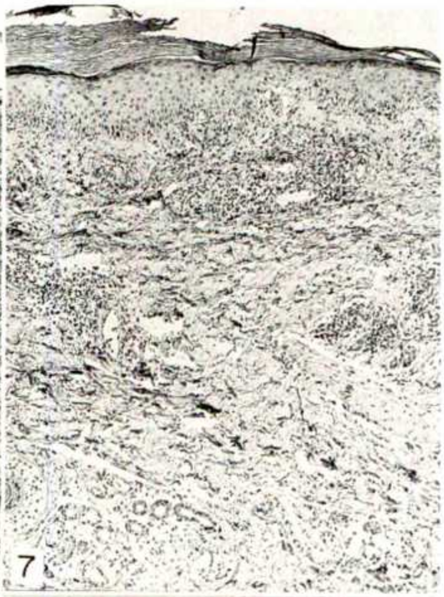
FIGURE 5. Dermal changes 4 days after application of 2 mg of DNCB corresponding with the 6th column of Fig. 2 (H & E, × 80).

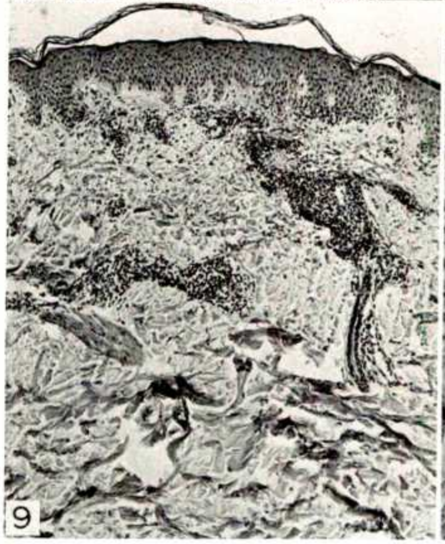
Day 13–*day* 17. The changes gradually disappear, the perivascular infiltrate diminishes as does the infiltrate around the dermal appendages (Fig. 7). The surface epithelium shows regeneration and is normal again by the end of day 7.

III. The group was sensitized with 2000 μg DNCB on the forearm. Fourteen days later challenge patch tests were performed on the back with 3, 10, 30, 50 and 90 μg DNCB.

After 48 h punch biopsies were taken from these challenge sites. Histologically, as shown in Fig. 8,







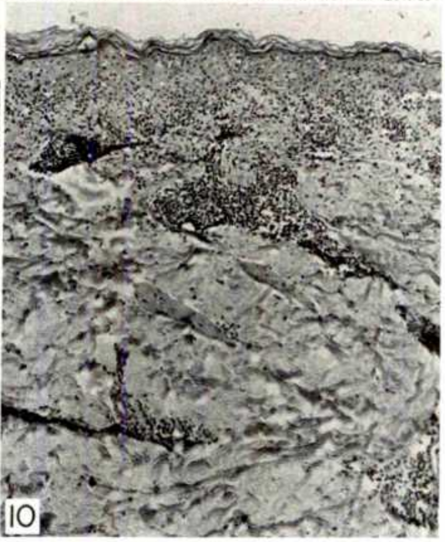


FIGURE 6. Dermal changes 12 days after application of 2 mg of DNCB corresponding with the 14th column of Fig. 2 (H & E, × 80).

FIGURE 7. Dermal changes after 15 days after application of 2 mg of DNCB corresponding with the 16th column of Fig. 2 (H & E, × 80).

FIGURE 9. Dermal changes after challenge of a sensitized skin with 10 μg of DNCB corresponding with the 3rd column in Fig. 8 (H & E, × 80).

FIGURE 10. Dermal changes after challenge of a sensitized skin with 30 μg of DNCB corresponding with the 4th column of Fig. 8 (H & E, × 80).

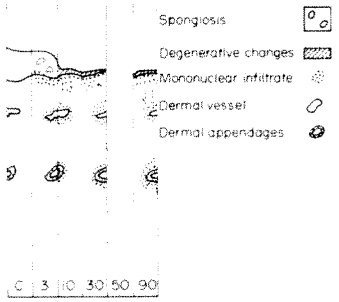


FIGURE 8. Schematic drawing of changes after dermal application of 3, 10, 30, 50 and 90 μg of DNCB with prior sensitization, in comparison to the control skin of the same patient (C).

The dermal changes are more pronounced in comparison to those seen in Fig. 1. Changes in the surface epithelium start after application of 3 μg and are more severe after application of increasing amount of DNCB. Already with 3 μg mononuclear infiltration at the dermal–epidermal junction can be observed as well as some perivascular infiltrate. After application of 10, 30 and 90 μg an extensive dermal infiltration of mononuclear cells as well as degenerative changes of the surface epithelium are observed.

application of 3, 10 or 30 μg of DNCB on the sensitized skin causes changes characterized by a perivascular dermal infiltrate which increases when more DNCB is applied. Also some infiltrate is seen around the dermal appendages and around the basal layers of the surface epithelium. The surface epithelium itself shows spongiosis with 3 μg DNCB and clear degenerative changes with 10 (Fig. 9) or 30 μg DNCB (Fig. 10). Such changes are even more marked when 90 μg of DNCB is applied. The histological effects of an application of 50 μg DNCB after prior sensitization could not be studied.

DISCUSSION

The results from the patch tests with DNCB on non-sensitized persons (group I) confirm the impression obtained in a former study (Bleumink *et al.*, 1974) that patch tests with DNCB may cause reactions of a primary irritant nature. These may hamper a correct reading of the challenge tests and cause false positive results. The results of the challenge tests with 3, 10 and 30 $\mu g/cm^2$ DNCB on a group of sensitized persons (group II), in Table 3, indicate furthermore that in all cases patch tests with 3 and/or 10 $\mu g/cm^2$ would have yielded sufficiently trustworthy results.

The one exception (no. 9) in group I, in which an unsensitized but eczematous person also reacted with an erythematous reaction to 3 and 10 μg DNCB/cm^2, may be explained by the results of Björnberg (1968). He proved that even a localized eczema provokes an increased irritability to toxic compounds at remote skin areas.

The histopathology of the reactions to DNCB in a non-sensitized and a sensitized group of persons gives an opportunity to compare purely toxic and combined toxic/allergic epidermal reactions to the same compound. In the non-sensitized skin (group I) histological changes appear after application of 30 μg or larger amounts of DNCB/cm^2. These initial alterations consist of a slight spongiosis in the superficial part of the epidermis. Perivascular infiltration and degenerative changes of the surface epithelium are observed only when larger amounts have been applied.

In sensitized healthy individuals (group III) application of 3 μg DNCB/cm^2 is sufficient to initiate histological changes: a perivascular infiltration in the dermis and a superficial spongiosis develop. Thus the principal difference between the reaction of the sensitized and non-sensitized skin appears to be primarily quantitative: there is a markedly increased reaction to lower amounts of DNCB in the sensitized epidermis. The nature of the reaction—spongiosis and perivascular mononuclear infiltration—however, is identical.

In group II the chain of reactions occurring after the application of a sensitization dose of 637 μg per cm^2 DNCB was studied over a period of 17 days. During this period the initial primary irritant reaction was followed in all but three persons by a more or less intense flare-up reaction, indicating that the intended sensitization had taken place. In two of these persons with a clinically absent flare-up reaction the challenge tests were also negative. These persons belong to the older age groups; decrease of DNCB reactivity with increasing age is a well-known phenomenon (Catalona *et al.*, 1972; Bleumink *et al.*, 1974). In the only other person with a macroscopically negative flare up, the challenge tests subsequently indicated a successful sensitization. (Histological examination of these negative flare ups has not been performed.) The biphasic reaction, occurring on days 1–5 and on days 8–17 after application of a DNCB sensitization dose, consists histologically of essentially the same components: spongiosis, degenerative changes of the epidermal layer, and mononuclear infiltration around dermal vessels and appendages penetrating into the epidermal layers. The only difference could be found in the longer duration of the second phase of the reaction.

In conclusion, in this study it was not possible to differentiate histologically between an allergic and a toxic reaction to DNCB. In order to avoid wrong interpretation of a toxic reaction, the amount of DNCB used for challenge testing must be the lowest dose that will elicit an allergic response. Our results lead to the conclusion that challenge patch tests with 3 and 10 μg DNCB/cm^2 yield sufficiently trustworthy results.

REFERENCES

BANDMANN, H.J. (1960) Beitrag zur Histopathologie allergischer epicutaner Testreaktionen. *Hautarzt*, **11**, 258, 310, 355, 393.

BANDMANN, H.J. & FREGERT, S. (1973) *Epicutantestung*, p. 28. Springer, Berlin.

BJÖRNBERG, A. (1968) *Skin reactions to primary irritants in patients with hand eczema*, pp. 138–142. Oscar Isacsons Tryckeris, Göteborg.

BLEUMINK, E., NATER, J.P., SCHRAFFORDT KOOPS, H. & THÉ, T.A. (1974) A standard method for DNCB sensitization testing in patients with neoplasms. *Cancer*, **33**, 911.

CATALONA, W.J., TAYLOR, T.P., RABSON, A.S. & CHRETIEN, P.B. (1972) A method for dinitrobenzene contact sensitization—A clinicopathological study. *New England Journal of Medicine*, **286**, 399.

EILBER, F.R. & MORTON, D.L. (1972) Impaired immunologic reactivity and recurrence following cancer surgery. *Cancer*, **25**, 362.

GROSS, L. (1965) Immunological defect in aged population and its relation to cancer. *Cancer*, **18**, 201.

HAXTHAUSEN, H. (1939) Some problems concerning the pathogenesis of allergic eczemas, elucidated by experiments on sensitization with dinitrochlorobenzene. *Acta dermato-venereologica*, **20**, 257.

LACHAPELLE, J.M. (1972) Comparative study of ^{3}H-thymidine labelling of the dermal infiltrate of skin allergic and irritant patch test reactions in man. *British Journal of Dermatology*, **87**, 460.

ROSTENBERG, A. & KANOF, N.M. (1941) Studies in eczematous sensitizations. I. A comparison between the sensitizing capacities of two allergens and between two different strengths of the same allergen and the effect of repeating the sensitizing dose. *Journal of Investigative Dermatology*, **4**, 505.

TURK, J.L. & WATERS, M.F.R. (1969) Cell-mediated immunity in patients with leprosy. *Lancet*, **ii**, 243.

WALKER, F.B., SMITH, P.D. & MAIBACH, H.I. (1967) Genetic factors in human allergic contact dermatitis. *International Archives of Allergy and Applied Immunology*, **32**, 453.

WILHELM, E., SARKANY, I. & CALNAN, C.D. (1958) Studies on Contact Dermatitis. *Transactions of the St John's Hospital Dermatological Society*, **41**, 31.

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Immunoglobulin and complement bearing polymorphonuclear leukocytes in allergic contact dermatitis and psoriasis vulgaris*

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SUMMARY

Immunofluorescence techniques were used to demonstrate the presence of immunoglobulin (Ig) and complement (C) bearing polymorphonuclear leukocytes (PMNL) in specimens of skin from the lesions of ten patients with allergic contact dermatitis and of ten with psoriasis vulgaris.

The distribution patterns of these Ig- and C-coated cells in the peripheral blood of the twenty patients were also studied. Ten normal healthy controls were included.

In cases of allergic contact dermatitis, IgD was the predominant class of Ig on the PMNL. The percentages of C-bearing PMNL were also significantly increased, suggesting that both molecules might be present on the cell membrane of these cells at the same time.

In psoriasis vulgaris, the predominant class of Ig on the PMNL was IgG. The percentages of PMNL coated with C were not significantly raised.

These results suggest that in allergic contact dermatitis the PMNL can be coated with both Ig and C which may render them cytophilic; they also show that in psoriasis vulgaris PMNL can bind Ig.

In the ten healthy controls, it was possible to distinguish between those PMNL which are and those which are not bearing immunoglobulin.

In previous studies (Cormane, Husz & Hamerlinck, 1974a), an increase in the number of circulating lymphocytes with characteristic receptors for anti-human immunoglobulin (Ig) and complement (C) factors has been reported in patients with certain varieties of dermatitis. It is now known that not only lymphocytes, but also macrophages, monocytes, mast cells and granulocytes participate in the immune response and related inflammatory processes. It is therefore reasonable to expect that some of the latter types of cells also may possess a comparable recognition mechanism. Receptors for C have been demonstrated on mouse polymorphonuclear leukocytes (PMNL) and peritoneal macrophages by Lay & Nussenzweig (1968). Moreover, using immunofluorescence (IF) techniques, we have seen Ig- and C-bearing PMNL as well as Ig- and C-coated lymphocytes in biopsies of lesional skin in certain varieties of dermatitis (Cormane *et al.*, 1974a).

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It is the purpose of this paper to describe the presence of receptor sites for Ig and C factors on the membrane of circulating leukocytes in patients with allergic contact dermatitis or with psoriasis. Psoriasis vulgaris was included in this investigation because Ig- and C-coated PMNL can be demonstrated especially in the Munro abscesses in that disease.

MATERIALS AND METHODS

Patients with allergic contact dermatitis (ten) and psoriasis vulgaris (ten) provided the PMNL. Normal healthy staff members (ten) served as controls.

Polymorphonuclear leukocyte separation

The PMNL were separated by the method of Böyum (1968). This procedure depends upon such properties of the cell-clumping mixture as its density, viscosity and osmolarity; it is further influenced by blood volume, dilution of blood, and temperature. Ten millilitres of venous blood was collected and defibrinated in Erlenmeyer flasks containing fifteen glass pearls.

After defibrination, 2 ml of isotonic NaCl solution was added. This suspension was layered on top of 40 ml Isopaque-Dextran mixture and left for 45 min; the erythrocytes are clumped by the Isopaque-Dextran at the interface and sediment by gravitation to the bottom of the tube. The plasma layer was aspirated then washed once in isotonic NaCl for 5 min at 200 *g*. The cells were resuspended in 8 ml isotonic NaCl and layered onto an Isopaque-Ficoll mixture. The latter suspension was centrifuged at 400 *g* for 20 min. The bottom of the tube then contained the PMNL. These cells were

TABLE 1. Characteristics of FTC-protein conjugates

Conjugates	Batch no.	FTC content (μg/ml)	Protein content (mg/ml)	F/P ratio (μg/mg)	Molar ratio (F/P × 0·41)	Antibody unitage	Working dilution
Rabbit antihuman* IgG/FTC	4572	26	9·6	2·7	1·11	2	1/40
Goat antihuman* IgA/FTC	6-171	31·04	32·8	0·94	0·38	4	1/40
Rabbit antihuman† IgM/FTC	KH 15-10-A2	81	14·2	5	2·0	2	1/40
Rabbit antihuman† IgD/FTC	KH 20-05-A2	48·75	10·4	4·6	1·9	2	1/40
Rabbit antihuman† IgE/FTC	KH 25-3-A2	34·8	11·2	3·1	1·27	—	1/40
Rabbit antihuman† β_1A/FTC	KH 41-9-P01	29·7	10·4	2·85	1·17	2	1/40
Rabbit antiswine* Ig/FTC	2-969	18·3	15·6	1·17	—	—	1/40

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FTC content of conjugates was determined by the method of McKinney, Pearce & Spillane (1966) using fluorescein diacetate (FDA) as reference standard. Protein content was measured by the method of Lowry *et al.* (1951). Antibody unigate conjugates was determined in a standard gel diffusion test using 1 mg/ml respective antigens, except in case of FTC/anti β_1A conjugate where normal serum was used as a source of antigen, against doubling dilutions of conjugates as described by Beutner, Sepulveda & Barnett (1968). A 1 mg/ml solution of human IgG, IgM, IgA and IgD contained 6·6, 129, 30·5 and 100 antigen units respectively in relation to international reference serum.

Staining properties and specifity of the class-specific conjugates (1–5) were tested in indirect IF in normal and monoclonal human bone marrow plasmacytes.

washed with isotonic NaCl three times at 200 *g* for 5 min. For the separation only thoroughly cleaned and siliconized glassware was used.

Immunofluorescence staining and examination

The chemical and immunochemical data of the conjugates used are listed in Table 1. Direct staining of cells in suspension was carried out by suspending 2×10^5 washed viable PMNL in 0·25 ml of the 1:40 diluted conjugate then incubating them at room temperature for 30 min. After incubation, the cells were twice washed for 5 min at 200 *g* in isotonic NaCl and then resuspended in 0·2 ml of the same solution.

Immunofluorescence examination was carried out on the suspension for immunoglobulins, and on a smear for the third component of complement. In each case the percentages of Ig- and C_3-positive PMNL were determined by examining the microscope field alternately with blue narrow band (480–490 nm) epi-illumination for fluorescence, and with conventional light.

Fluorescing cells, in most instances, showed a patchy, coarsely grained staining. Trypan Blue viability of the cell preparations used was performed before IF staining and was greater than 94–96%.

Specificity test

Fluorescein conjugated rabbit anti-swine (RASw/FTC) leukocyte antiserum failed to stain the cells in suspension. Adding normal rabbit serum to the cell suspensions did not reduce the numbers of cells staining. Unconjugated antiserum specifically inhibited staining by its counterpart in the conjugated antiserum but did not inhibit staining by conjugated antiserum directed against a different class of Ig.

Microscopy

A Leitz Orthoplan microscope with a Xenon XBO-75 lamp and a Leitz Vertical illuminator were used. FTC fluorescence was detected with the filter combination: BG 38 (4 mm), KP 490 (2 mm), GG 475 (2 mm) and a secondary filter OG 515 (3 mm). In all cases the examination was performed with Leitz water immersion objectives (× 50 and × 90).

Statistics

Statistical analysis was performed according to the Student's '*t*' test with pooled variance. Differences were accepted under a significant 5% level.

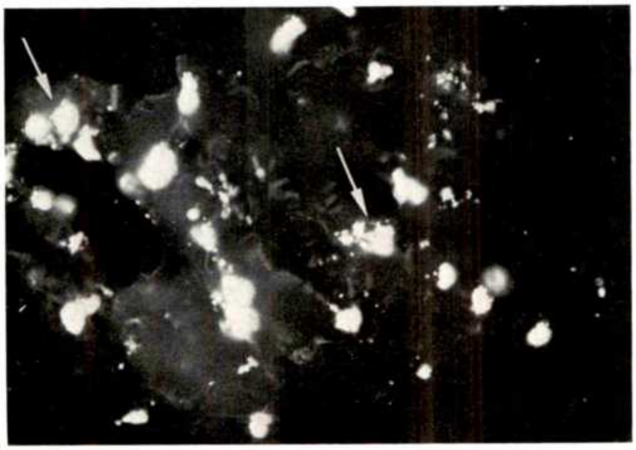

FIGURE 1. Cryostat section of a lesional skin specimen from a patient with allergic contact dermatitis. DIF: RAHuIgD/FTC (unitage 2, dilution 1:40). (Blue narrow-band epi-illumination, × 300.) Note the coarsely grained IgD positive PMNL.

RESULTS

In ten healthy controls (Table 2) the mean percentages of IgG-, IgA-, IgM-, IgD- and IgE-bearing PMNL were respectively: 1·9, 2·6, 5·8, 5·1 and 4·8%. The ranges respectively were: 0·5–6·0% for IgG; 0·5–7·0% for IgA; 2·0–9·0% for IgM; 2·0–9·3% for IgD; and 1·0–10·2% for IgE. The mean percentage of C_3-coated cells was 6·8% with a range of 3·0–12·0%.

TABLE 2. Ig- and C_3-determinants on granulocytes in ten healthy controls

Patient no.	IgG%	IgA%	IgM%	IgD%	IgE%	C_3%
1	1·0	2·0	4·0	2·0	1·0	5·0
2	1·5	5·0	4·0	7·5	5·0	7·3
3	1·5	3·0	7·5	6·0	7·0	7·0
4	1·5	0·5	7·3	3·0	1·5	6·0
5	1·0	1·3	6·0	5·6	6·3	6·5
6	2·4	3·8	7·8	6·5	3·0	3·0
7	3·0	2·6	7·5	9·3	10·2	8·0
8	6·0	7·0	9·0	7·0	8·0	12·0
9	0·5	0·5	2·0	2·0	3·0	4·0
10	0·5	0·5	2·5	2·5	2·5	9·0
Range	0·5–6·0	0·5–7·0	2·0–9·0	2·0–9·3	1·0–10·2	3·0–12·0
Mean value	1·9	2·6	5·8	5·1	4·8	6·8

In ten patients with allergic contact dermatitis (Table 3), IgD was the predominant class of the Ig-bearing PMNL, with a mean of 13·0% and a range of 9·0–17·0%. The mean percentage and range of C_3-positive cells were 22·7% and 10·0–40·0%.

TABLE 3. Ig- and C_3-determinants on granulocytes in ten patients with allergic contact dermatitis

Patient no.	IgG%	IgA%	IgM%	IgD%	IgE%	C_3
1	5	4	8	11	10	16
2	4	0·5	2	14	12	14
3	1	0	10	9	1	11
4	1·5	3	9·5	17	13	37
5	4	2	11	15	14	40
6	8·3	1	4	14	2	12
7	2·5	4	3	11	6	18
8	2	2	3	11	9	10
9	5	1	4	14	6	17
10	6	4	6	14	7	10·5
Range	1·5–8·3	0–4	2–11	9–17	2–14	10–40
Mean value	4·0	2·2	6·1	13·0	8·0	22·7

In ten patients with psoriasis vulgaris (Table 4), the percentages of Ig-bearing PMNL were slightly higher for all Ig classes except IgD than in the healthy controls. The mean percentages of IgG-, IgA-, IgM-, IgD- and IgE-bearing cells were respectively 7·6, 4·8, 7·9, 5·3 and 6·7% with ranges of 1·0–18·0% for IgG; 1·0–16·0% for IgA; 4·5–16·0% for IgM; 2·0–10·2% for IgD; and 2·0–12·5% for IgE. The mean percentage of C_3-coated cells was 7·2% with a range of 2·5–15·0%.

TABLE 4. Ig- and C_3-determinants on granulocytes in ten patients with psoriasis vulgaris

Patient no.	IgG%	IgA%	IgM%	IgD%	IgE%	C_3%
1	2·0	3·0	4·5	7·0	2·0	7·0
2	14·5	6·4	4·0	4·0	3·0	3·0
3	16·6	1·0	12·6	4·5	3·0	7·0
4	5·4	1·1	6·0	4·0	11·0	12·0
5	5·3	7·7	5·0	8·9	4·0	9·0
6	18·0	2·0	8·0	4·3	12·5	15·0
7	1·0	1·0	8·0	2·0	5·0	2·5
8	8·0	5·4	16·0	5·5	8·2	7·3
9	4·2	16·0	8·0	10·2	12·0	4·0
10	1·0	4·0	7·0	3·0	6·0	5·0
Range	1·0–18·0	1·0–16·0	4·5–16·0	2·0–10·2	2·0–12·5	2·5–15·0
Mean value	7·6	4·8	7·9	5·3	6·7	7·2

DISCUSSION

Fluorescein-conjugated monospecific antisera to the five main heavy-chain classes of Ig and to complement were used for direct IF staining of cryostat sections taken from the untreated lesional skin of ten patients with allergic contact dermatitis and of ten patients with psoriasis. The procedure revealed not only fluorescing lymphoid cells as reported by Cormane *et al.* (1974a) but also PMNL-like cells with a coarsely grained fluorescing pattern respectively.

In allergic contact dermatitis, the predominant Ig class carried by these PMNL-like cells appeared to be IgD (Fig. 1), whereas in psoriasis there was no predominant class.

These observations suggest that PMNL participate in the underlying mechanisms in allergic contact dermatitis and in psoriasis.

As it is sometimes very difficult to identify the fluorescing cells in skin specimens by IF techniques (Cormane, 1974), it was decided to find out whether the presence of fluorescing PMNL-like cells could be related to the distribution pattern of the circulating PMNL in the patients with allergic contact dermatitis and in those with psoriasis. Ten healthy controls were also included in the study. The PMNL were isolated by the method of Böyum (1968).

It is noteworthy that there were circulating Ig- and C-bearing PMNL in the healthy controls (Table 2). The mean percentages of the Ig-bearing PMNL appear to be less than 6% or so, whereas that of the C-bearing PMNL in the controls was only slightly higher (6·8%).

In all ten patients with allergic contact dermatitis, the predominant class of Ig on the circulating PMNL was invariably found to be IgD ($P<0\cdot005$). However, the percentages of IgE-bearing PMNL were also increased in most of the patients, although not so much ($P<0\cdot05$) as the IgD-positive cells were (Table 3).

In allergic contact dermatitis there is a striking similarity between the distribution patterns of PMNL and of lymphocytes (Cormane *et al.*, 1974a; Cormane, 1974).

In eight of the ten patients with allergic contact dermatitis, the percentages of C_3-bearing and of IgD-coated PMNL were raised to a similar degree ($P<0\cdot005$); this suggests that both molecules are present concomitantly on the cell membrane of the PMNL. In two of the ten patients (Nos 4 and 5), in whom the percentages of IgE-bearing PMNL were significantly increased, much elevated values for C_3-coated PMNL were also found.

These findings may indicate the presence of immune complexes on the cell membrane of PMNL rendering them cytophilic and more active (Nydegger *et al.*, 1973).

In psoriasis, the mean values and the ranges of the percentages of circulating PMNL coated with IgA, IgM, IgE and C_3 are not significantly higher than the corresponding values for the healthy controls (Table 4), and the figures for the IgD-bearing circulating PMNL are also similar to those of the controls. As a class, the IgG-coated PMNL, however, seem to predominate, the differences and the values for the controls found for these cells being statistically significant ($P<0{\cdot}005$).

The percentages of C_3 bearing PMNL show no significant increase in the psoriasis patients with two exceptions (patients 4 and 6).

It is tempting in the light of these results to assume that, in allergic contact dermatitis and psoriasis, antibody is present on the cell membrane of circulating PMNL. This assumption could easily be proved by means of elution experiments (Cormane, Husz & Hamerlinck, 1974b).

In psoriasis, such experiments have already been done and have shown that antibodies can be eluted from the membrane of the PMNL and that they are directed mainly against the active nuclei of the epidermis of clinically uninvolved skin (Cormane, 1975; Cormane, Hunyadi & Hamerlinck, 1975).

A comparison between allergic contact dermatitis and psoriasis seems justifiable since the initial psoriatic lesion shows a striking resemblance to a delayed type of allergic skin reaction (Braun-Falco & Christophers, 1974).

The experiments carried out in this study favour the concept that PMNL play an active role in the pathogenesis of both dermatoses. The main difference to be found with regard to this category of cells is the predominance of IgD-bearing PMNL in allergic contact dermatitis on the one hand, and of IgG-bearing PMNL in psoriasis on the other.

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REFERENCES

BEUTNER, E.H., SEPULVEDA, M.R. & BARNETT, E.V. (1968) Quantitative studies of immunofluorescent staining. *Bulletin of the World Health Organization*, **39**, 587.

BÖYUM, A. (1968) Separation of leukocytes from blood and bone marrow. *Scandinavian Journal of Clinical and Laboratory Investigation*, **21** (Suppl.), 97.

BRAUN-FALCO, O. & CHRISTOPHERS, E. (1974) Structural aspects of initial psoriatic lesions. *Archives of Dermatological Research*, **251**, 95.

CORMANE, R.H., HUSZ, S. & HAMERLINCK, F. (1974a) Immunoglobulin- and complement-bearing lymphocytes in allergic contact dermatitis and atopic dermatitis. *British Journal of Dermatology*, **90**, 597.

CORMANE, R.H., HUSZ, S. & HAMERLINCK, F. (1974b) Elution of antibodies from the lymphocyte membrane in certain dermatoses. *British Journal of Dermatology*, **91**, 315.

CORMANE, R.H. (1974) B and T cells in dermatitis. *Mayo Clinic Proceedings*, **49**, 531.

CORMANE, R.H. (1975) Immunological mechanisms in psoriasis. Presidential address at the occasion of the second joint meeting of the European Society for Dermatological Research, Amsterdam.

CORMANE, R.H., HUNYADI, J. & HAMERLINCK, F. (1975) Les mécanismes immunologiques de Psoriasis. Communication présentée à l'occasion des Journées Nationales de la Société Francaise de Dermatologie auront lieu à Montpellier les 23, 24 et 25 Mai.

LAY, W.H. & NUSSENZWEIG, V. (1968) Receptors for complement on leukocytes. *Journal of Experimental Medicine*, **128**, 991.

LOWRY, O.H., ROSENBROUGH, N.J., FARR, A. & RANDALL, R.J. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265.

MCKINNEY, R.M., PEARCE, G.W. & SPILLANE, J.T. (1964) Fluorescein diacetate as a reference color standard in fluorescent antibody studies. *Analytical Biochemistry*, **9**, 474.

NYDEGGER, U.E., ANNER, R.M., GEREBTZOFF, A., LAMBERT, P.H. & MIESCHER, P.A. (1973) Polymorphonuclear leukocyte stimulation by immune complexes. Assessment by nitroblue tetrazolium dye reduction. *European Journal of Immunology*, **3**, 465.

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A case of *Pyemotes* dermatitis

WITH A NOTE ON THE ROLE OF THESE MITES IN SKIN DISEASE

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SUMMARY

Acute dermatitis developed in a fisherman after contact with old cherry-wood. A *Pyemotes* mite, probably *P. beckeri*, found in the wood, was thought to be responsible—thus illustrating the importance of appropriate laboratory examinations for ectoparasites. Human skin eruptions caused by indigenous, in contrast to imported, *Pyemotes* species have not previously been reported in Britain. These tarsonemoid mites attack small insect hosts and their possible role in dermatoses is discussed. Dermatitis caused by these mites is probably world-wide in distribution, but during the last century the confusing acarological nomenclature surrounding *Pyemotes* has resulted in an equally confusing variety of dermatological diagnoses.

CASE REPORT

The patient, a fisherman aged 46 years, makes his own crab-pots. In 1970, he changed from the traditional, round 'withies' crab-pots made from willow to the barrel-like 'Frenchman' pots made from lathes of cherry-wood tied with nylon at the ends. During the first year, when he used newly cut local cherry-wood, he remained well. However, in October 1971, for the first time, he used cherry-wood cut during the previous season. Within 24 h of using this, he developed a severe, intensely irritating, papular erythematous rash distributed over the head, neck, face, abdomen and limbs—especially on the hands and forearms; his eyelids also became swollen. This episode lasted for about 3 days and then quickly regressed. Subsequently he observed that similar episodes recurred on making crab-pots from the old cherry-wood. They also occurred after working or brushing-up in his shed if he caused much dust to rise. Clinical examination during one such episode revealed features typical of an acute allergic contact dermatitis.

Until 31 years old, this patient had been a farm-worker, but had given this up because of respiratory and cutaneous hypersensitivity to mouldy hay. Later he kept pigs, but had to abandon this because the dust from pig meal produced an irritating rash on his head, neck and hands. There was also a strong family history of allergy; his mother suffered from hay fever, his sister from asthma, one son suffers from allergic rhinitis when in contact with hay and barley, and another son suffers from bronchospasm when in damp, musty rooms.

Laboratory investigations

Strips of cherry-wood, half-round and about 2 feet long × 1 inch wide, one of old and the other of new wood, were submitted for examination. There was fungal growth visible to the naked eye on the old cherry-wood; it yielded a *Penicillium* species on culture. Loose bark and all crevices or broken pieces of wood were scrutinized with a low-power stereoscopic microscope, but nothing unusual was seen. However, as the laboratory was carrying out work on the isolation of ectoparasites from the personal environment of patients, some loose bark and crevices were probed with a bacteriological wire loop wetted with parasite examination fluid, and, unexpectedly, mites were detected in the old cherry-wood. All loose bark was then stripped off each piece of wood, and the bark and wood strips each placed in separate plastic bags, and washed twice with 20 ml of parasite 'fixing' solution. After shaking, each lot of fluid was poured into 1 oz universal containers, slowly centrifuged and examined by pipetting the deposit onto clean microscope slides. After drying at room temperature, these were examined microscopically and suspicious material transferred to parasite examination fluid on slides; the procedures and solutions used for fixing and examining ectoparasites were described by Hewitt *et al.* (1973).

Numerous preparations of mites were made on slides, and two species present in the old cherry-wood were subsequently identified by acarological procedures. One of these mites was *Calvolia heterocoma*, a sarcoptiform mite not previously associated with human or animal dermatoses. The other was a *Pyemotes* species, probably *P. beckeri* (Figs 1 and 2).

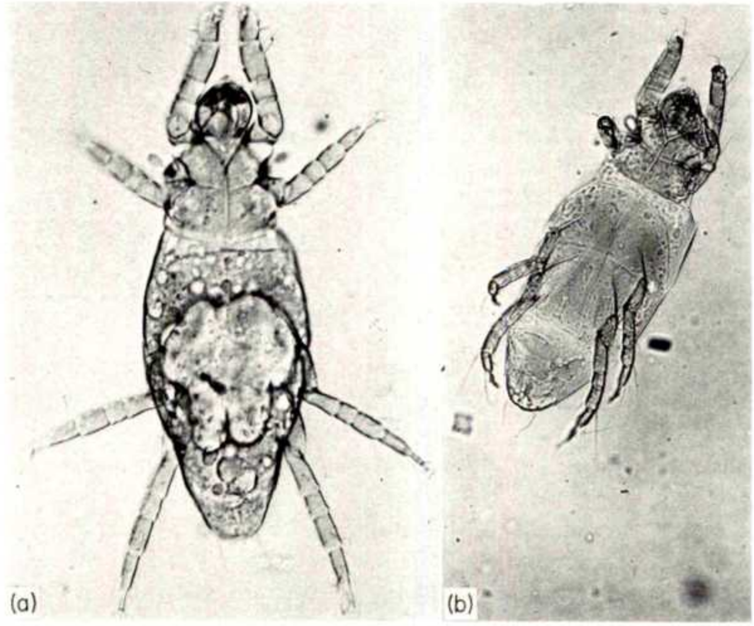

FIGURE 1. *Pyemotes beckeri* Krczal. Female (a) dorsal view, (b) ventral view. Actual size c. 280 μm.

Clinical investigations

Patch tests were performed on the patient with the following antigens prepared in physiological saline: powder and scrapings from (a) old and (b) new cherry-wood; (c) live and (d) autoclaved suspensions from cultures of the *Penicillium* mould grown on Sabouraud's medium. The *Penicillium* mould suspensions and the new cherry-wood produced no reaction, but the old wood, which contained mites, gave a strongly positive reaction. Prick tests with the usual commercial antigens gave a strong reaction with house dust mite extract, as well as other minor reactions. Unfortunately it was not possible to investigate the patient further to exclude allergy to lichens, or to perform patch or prick tests with the actual mites isolated. However, the circumstantial evidence strongly suggested that the *Pyemotes* mite was responsible for his skin trouble which recurred the following season when he

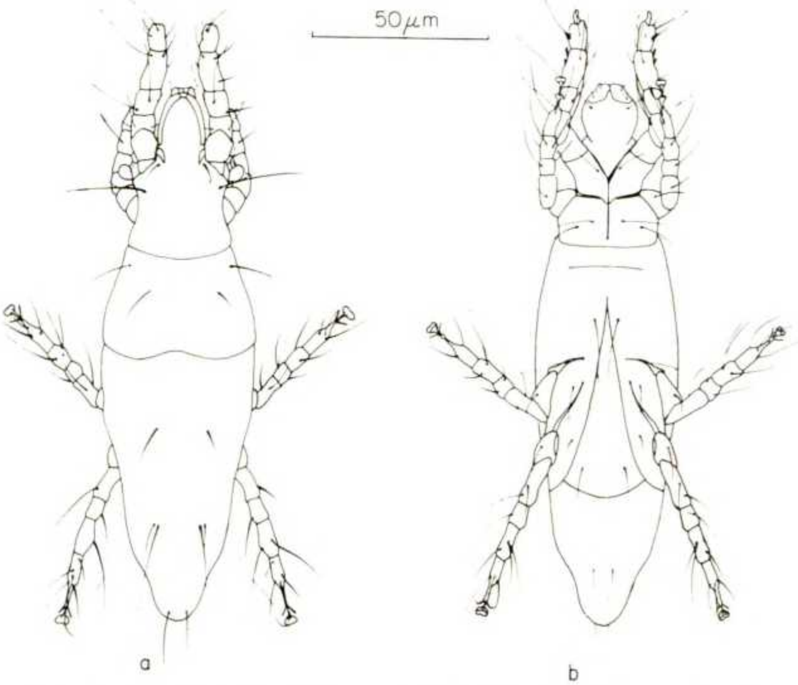

FIGURE 2. Line drawing of *Pyemotes beckeri* (a) dorsal view, (b) ventral view.

resumed work. Although the natural insect host of the *Pyemotes* mite was not found, it was assumed to be the larval stage of the woodworm or furniture beetle *Anobium punctatum*, which is very common in Cornwall and other parts of Britain, both indoors and outside.

DISCUSSION

The rapid acute onset of the cutaneous eruption after contact with old cherry-wood suggested an immediate weal Type I allergic reaction to the *Pyemotes* mite, combined perhaps with an irritant effect. The positive patch test suggested a Type IV delayed hypersensitivity reaction. Although it is impossible to assess now, mites in the environment, including those in hay and in pig meal, could have played a role in this patient's earlier allergic disorders, as well as in those of his family.

Pyemotes species of tarsonemoid mites are probably world-wide in distribution (Krczal, 1959). The attention they have received in both medical and zoological literature is reflected in the many different names they have been given, and these in turn reflect how often they have been 'discovered' for the first time. All *Pyemotes* species are small, virgin females varying from about 200 to 300 μm in size; males are much smaller and live on the bodies of females. When gravid, females become very large, some reaching as much as 2 mm in size. The young are born alive and sexually mature, females greatly outnumbering males. The adult males living on gravid females assist with birth, pulling the young from the parent body and then mating with newborn females immediately.

Pyemotes species are all primarily parasites of insects or their larvae, and they only affect man or other animals if the plant material on which their natural hosts feed happens to come into contact in some way with the skin (Fig. 3). Wood, straw, grain, cotton seeds and certain dried vegetables, which are often infested with insect pests, may thus contain vast numbers of these tarsonemoid mites. They feed on the egg, larval, pupal or even the adult stages of various insects by piercing the soft integument with their stylet-like mouth parts and sucking them dry (Fig. 4). The insect stages attacked vary with different species of *Pyemotes* mites.

The distribution and actual identity of *Pyemotes* species involved in dermatoses is confusing and difficult to assess. Many different names were given to *Pyemotes* species before 1886; subsequently, rightly or wrongly, until the masterly work of Oudemans (1937a, b) they were virtually all called *P. ventricosus* in medical and zoological literature. Indeed, even some recent dermatological works still refer to '*P. ventricosus*' as the cause of grain or straw itch (Fine & Scott, 1963, 1965; Rook, Ebling & Wilkinson, 1968). In fact, this species was first described by Newport (1853) who observed them parasitizing his bees. Despite imprecise definition, *P. tritici*, first described by Lagrèze-Fossot

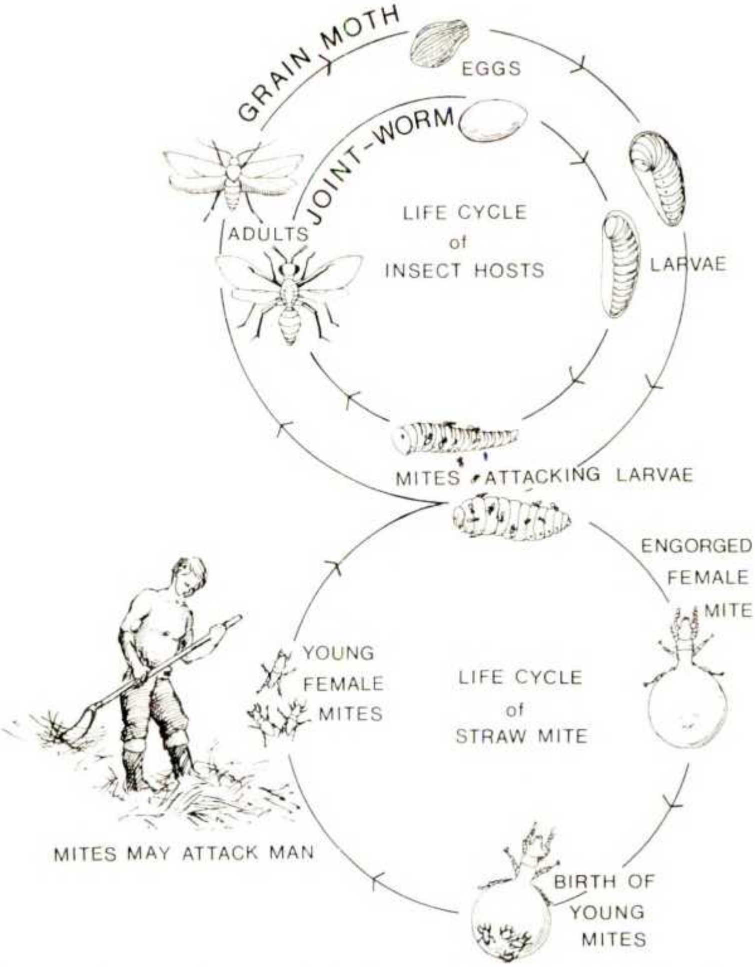

FIGURE 3. Life cycle of *Pyemotes tritici* and its insect hosts. (After Booth & Jones, 1954.)

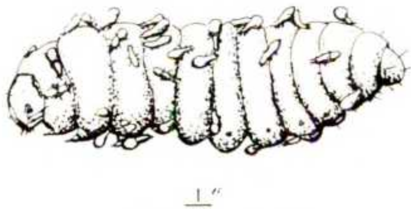

FIGURE 4. Caterpillar of Angoumous Grain Moth infested with *Pyemotes tritici*. (After Swan, 1937a.)

& Montané (1851) as the cause of human skin irritation during harvesting, is the species which commonly parasitizes the pests of grain, seeds, straw, and other material of vegetable origin—particularly the various stages in the life cycle of the Angoumois Grain Moth (*Sitotroga cerealella*), the Wheat Joint Worm (*Harmolita tritici*) and the Cotton Seed Moth (*Pectinophora gossypiella*). As would be expected, *P. tritici* is therefore probably the species actually concerned in many of the cases and epidemics of occupational dermatoses. The world-wide distribution of both indigenous and imported *Pyemotes* mites associated with skin disease, as well as some of the descriptive clinical terms previously used, are given in Tables 1 and 2. Thus O'Connor (1920) described how men unloading cotton seed from Egypt refused to work because of the resulting intense irritation of the skin. Examination of the seeds revealed the small pink maggots of the Cotton Seed Moth, on which he subsequently observed large numbers of mites, often in large masses. A large outbreak of dermatitis, involving some 1700 patients at Indiana State Fairs, was described by Booth & Jones (1952). Although they attributed this outbreak to a *Pyemotes* species infesting straw used for animals, there were, however, other clinical features including headache, pyrexia, diarrhoea and vomiting.

Clinically, Schamberg (1910) noted three kinds of skin eruption associated with *Pyemotes* mites:

TABLE 1. Distribution of some *Pyemotes* mites associated with skin eruptions

Country	Material	Reference
Algeria	Sacks of grain	Nouffert (1875)
Algeria	Cereals	Gros (1908)
Algeria	Barley	Sergeant & Alary (1916)
Australia	Hay	Swan (1934)
Austria	Grain imported from lower Hungary	Geber (1879)
Britain	Cotton seed from Egypt	O'Connor (1920); Willcocks (1914); Nixon (1915)
Britain	Barley from Morocco	O'Donovan (1922); Askins (1924); Thompson (1925)
Britain	Wood	Hickin (1969)
Bulgaria	Grain	Jurukoff (1937)
Egypt—see Britain		
France	Sacks of wheat	Lagrèze-Fossot & Montané (1851)
France	Grain from Tunisia	Bourgain & Ardisson (1966)
Germany	Wheat from Russia	Koller (1882)
Germany	Barley in ports	Lindner (1903)
Hawaii	Wood	Arnold & Haramoto (1962)
Istanbul	Barley	Hodara (1921)
Italy	Grain imported from Albania	Ducrey (1909); Pantaleoni (1920); Savinetti (1926); Mariani (1952)
Japan	Grain	Aoki & Hasegawa (1966)
Morocco—see Britain		
Palestine	Hay	Katzenellenbogen (1947)
Roumania	Wheat from Russia	Flemming (1884)
Russia—see Germany and Roumania		
Sardinia	Grain	Idini (1926)
Spain	Straw	Dorado & Vidal (1946)
Switzerland	Straw and hay mattresses	Lutz (1939)
Tunisia—see France		
Turkey	Straw mattresses	Kennedy (1924)
U.S.A.	Straw	Goldberger (1910); Schamberg (1910); Webster (1910); Booth & Jones (1952); Micks (1963);
U.S.A.	Wood	Fine & Scott (1965)

TABLE 2. Some names given to skin lesions associated with *Pyemotes* mites

Name	Reference
Acarodermatitis urticarioides	Schamberg (1910); Kennedy (1924); Katzenellenbogen (1947)
Acariasi da grano	Pantaleoni (1920); Majocchi (1922)
Barley itch	Willis (1909); Thompson (1925)
Cotton seed dermatitis	Nixon (1915)
Dermatitis ditropenotus aureoviridis	Rawles (1909)
Dermatitis urticarioides parasistica	Stelwagon (1921)
Dermatitis schambergi	Goldberger (1910)
Gale des céréales	Mandoul *et al.* (1950)
Grain itch	Schamberg (1910); Booth & Jones (1952); Arnold & Haramoto (1962)
Grain-mite dermatitis	Stelwagon (1921)
Grain shovellers' itch	Rook *et al.* (1968)
Hay itch	Swan (1934)
Klawe itch	Erhorn (1913)
Lumbermen's itch	Stelwagon (1921)
Mattress itch	Stelwagon (1921)
Millers' itch	Thompson (1925)
Ohio scratches	Stelwagon (1921)
Prairie itch	Stelwagon (1921)
Schamberg's disease	Krczal (1959)
Straw dermatitis	Stelwagon (1921)
Straw itch	Rawles (1909)
Straw itch mite dermatitis	Fine & Scott (1965)
Straw mattress disease	Krczal (1959)
Straw packers' itch	Stelwagon (1921)
Swamp itch	Stelwagon (1921)
Texas mange	Stelwagon (1921)

urticarial–vesicular–papular; varicelliform; and erythema multiforme; but he described the essential lesions as weals capped by pin-point vesicles. Fine & Scott (1963, 1965) also described discrete erythematous papular urticaria with tiny vesicles and occasional central haemorrhagic puncta. In our case, the patient presented with a sheet-like papular erythematous rash on exposed areas, but no vesicles were seen.

Krczal (1959) recognizes twenty different species of *Pyemotes*, of which four have been reported in Britain. Apart from *P. tritici*, *P. boylei* and possibly *P. beckeri* in the present instance, none of the other seventeen species have been clearly associated with dermatoses; yet many of them, readily differentiated by their morphology, can attack numerous insects, including pests of grain and wood. It seems likely therefore that skin eruptions associated on environmental or occupational grounds with various materials subject to infestation by insect pests may well be caused by other *Pyemotes* species. For example, Fine & Scott (1963, 1965) described cases of typical 'straw itch mite dermatitis' caused by a *Pyemotes* species parasitizing the common furniture beetle, *Anobium punctatum*, although they called the mite '*P. ventricosus*'. This association between skin lesions and infested woodworm grubs lends further weight to the suggestion of Stelwagon (1921) that Lumbermen's itch might be due to *Pyemotes* mites. Indeed, in a survey in Britain, Hickin (1969) found that up to 60% of woodworm larvae in damp situations may be infested with *Pyemotes* mites—which he considered were the cause of some skin irritation in pest-control workers dealing with affected timber.

Although inconclusive, the present case again illustrates the importance of both clinical awareness and laboratory examination for mites in selected material from the personal environment of patients with unexplained dermatoses, particularly since such mites are rarely found on patients themselves. It also illustrates the importance of identification of ectoparasites.

ADDENDUM

Since this paper went to press, J.C.Moser of Louisiana has published his work 'Biosystematics of the straw itch mite with special reference to nomenclature and dermatology' (*Transactions of the Royal Enterological Society, London*, **127**, 185). He accepts that the correct name of this mite is *Pyemotes tritici* and that several other species occur. His experiments show that they are of varying toxicity to man, and therefore that precise identification is important, especially as it is suggested that they could be used for the control of such bark beetles as those that carry Dutch Elm Disease fungus. He found that *P. beckeri* was particularly toxic to its insect hosts, and that another species *P. schwerdfegeri*, also now known to occur in Britain on the Furniture Beetle, caused a milder dermatitis in man than that produced by *P. tritici*. He stresses the need to examine more material from Britain.

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REFERENCES

Aoki, J. & Hasegawa, K. (1966) On an epidemic of grain itch in barley storehouses in the central part of Japan, and mites concerned. *Japanese Journal of Sanitary Zoology*, **17**, 28. Japanese (English summary).

Arnold, H.L. & Haramoto, F.H. (1962) 'Grain itch' following fumigation for termites. *Dermatologia Tropica*, **1**, 37.

Askins, R.A. (1924) Dermatitis caused by *Pediculoides ventricosus*. *British medical Journal*, **ii**, 950.

Booth, B.H. & Jones, R.W. (1952) Epidemiological and clinical study of grain itch. *Journal of the American Medical Association*, **150**, 1575.

Bourgain, M. & Ardisson, H. (1966) Epidémies de dermatite prurigineusa à acariens libres d'aspect sarcoptoide des familles Tyroglyphidae et Pediculoididae à Toulon. *Bulletin de la Société de pathologie exotique*, **58**, 1154.

Dorado, F.G. & Vidal, J.M.C. (1946) Parasitsimo cutaneo por el *Pediculoides ventricosus*. *Revista Iberica de Parasitologia*, **6**, 293.

Ducrey, A. (1909) Acariasi da grano in forma epidemica dovuta al *Pediculoides ventricosus*. *Atti della Società toscana (fiorentina) d'igiene*, **24**, 42.

Erhorn, E.M. (1913) Notes and exhibitions. *Proceedings of the Hawaiian Entomologists' Society*, **2**, 206.

Fine, R.M. & Scott; H.G. (1963) Straw itch mite dermatitis caused by *Pyemotes ventricosus*. *Journal of the Medical Association of Georgia*, **52**, 162.

Fine, R.M. & Scott, H.G. (1965) Straw itch mite dermatitis caused by *Pyemotes ventricosus;* comparative aspects. *Southern Medical Journal*, **58**, 416.

Flemming, J. (1884) Über eine geschlechtsreife Form der als *Tarsonemus* beschreibenen Tiere. *Zeitschrift für gesamte Naturwissenschaften Halle*, **57**, 472.

Geber, E. (1879) Entzündliche Processe de Haut, durch eine bis jetzt nicht besimmte Milbe veranlabt. *Wiener medizinischer Presse*, **20**, 1361 & 1395.

Goldberger, J. (1910) The straw itch (dermatitis schaembergi); a disease new to American physicians. *Public Health Reports, Washington*, **25**, 779.

Gros, H. (1908) Sur un acarien parasite des teignes des céréales et cause accidentale d'eruptions cutanées ches l'homme. *Bulletin de la Société de pathologie exotique*, **1**, 249.

Hewitt, M., Barrow, G.I., Miller, D.C., Turk, F. & Turk, S. (1973) Mites in the personal environment and their role in skin disorders. *British Journal of Dermatology*, **89**, 401.

Hickin, N.E. (1969) The woodworm. *Science Journal*, **3**, 64.

Hodara, M. (1921) Epidemie einer durch Gerstenstaub hervorgerufenen erythemato-vesiculosen hauterkrankung. *Archiv für Dermatologie und Syphilis*, **130**, 325.

Idini, F. (1926) *Pediculoides ventricosus. Revista sud-americana de endocrinologie, immunologia y quimioterapia*, **9**, 513 & 615.

Jurukoff, B. (1937) Massenhafte Erkrankungen beim Menschen hervorgerufen durch Pediculoides ventricosus. *Archiv für Schiffs und Tropenhygiene unter bes. Ber. d. Pathologie und Therapie*, **41**, 337.

Katzenellenbogen, I. (1947) Acarodermatitis Urticarioides (Grain Itch). *Archiv für Dermatologie und Syphilis*, **55**, 621.

Kennedy, J.C. (1924) Acarodermatitis Urticarioides (Grain Itch) in Turkey. *Journal of the Royal Army Medical Corps*, **43**, 45.

Koller, J. (1882) Eine Getreidemilbe als Krankheitserregerin. *Pester medizinisch-chirurgische Press*, **36**, 127.

Krczal, H. (1959) Systemik und Ökologie der Pyemotiden. In: *Beiträge zur Systematik und Ökologie Mitteleurpaischer Acarina* (Ed. by H.J. Stammer), Bd. 1 Teil 2, p. 385.

Lagréze-Fossot, A. & Montané R.J. (1851) Observations microscopiques sur la mite du ble. *Recueil agronomique de la Société des sciences d'agriculture et belles lettres de Tarn-et-Garonne*, **32**, 37.

Lindner, P. (1903) Lästige Hauterkrankungen durch Milben bei Gersteabladungen. *Wochenschrift für Brauerei*, **20**, 548.

Lutz, W. (1939) Ein wahrscheinlich durch milbenhaltiges Stroh hervorgerufenes Exanthem bei Soldaten. *Schweizerische medizinische Wochenschrift*, **20**, 1233.

Majocchi, D. (1922) De un'epidemia di acariasi da grano. *Giornale di medicina militare*, **70**, 301.

Mandoul, R., Lepigre, A.L. & Jacqueman (1950) Apropos d'une nouvelle manifestation en Algérie de la gale des céréales à *pédiculoides ventricosus* Newport. *Algérie Médicale*, **6**, 433.

Mariani, G. (1952) Die episodi d'infestasione da *Pediculoides ventricosus* nel Bresciano. *Revista italiano Igiene*, **12**, 1.

Micks, D.W. (1963) An outbreak of dermatitis due to the grain itch mite, *Pyemotes ventricosus* Newport. *Texas Reports on Biology and Medicine*, **20**, 221.

Newport, G. (1853) Further observations on the habits of *Monodontomerus;* with some account of a new Acarus (*Heteropus ventricosus*) a parasite in the nests of *Anthophora retusa. Transactions of the Linnean Society of London*, **21**, 95.

Nixon, J.A. (1915) Cotton-seed dermatitis and its cause, *Pediculoides ventricosus. Bristol Medico-Chirurgical Journal*, **33**, 73.

Nouffert, - (1875) 'L'Acarus urticans' nouveau parasite de l'homme. *Bulletin de la Société des Séances physique naturelles et climatologiques del Algerie* (1875), 71.

O'Connor, F.W. (1920) An outbreak of itch due to a predaceous mite, occurring in England amongst men engaged in unloading cotton seed from Egypt. *Transactions of the Royal Society of tropical Medicine*, **13**, 10.

O'Donovan, W.J. (1922) Discussion on occupational dermatitis. *British Medical Journal*, **ii**, 499.

Oudemans, A.C. (1937a) *Kritisch historisch Overzicht der Acarologie.* Bd.C. Brill, Leiden.

Oudemans, A.C. (1937b) Over de veelheid der sorten van *Pediculoides. Tijdschift voor entomologie*, **80**, 4.

Pantaleoni, P. (1920) Vasta epidemia di acariasi da grano in Romagna. *Policlinico*, **27**, 1275

Rawles, L.T. (1909) Dermatitis Ditropenotus Aureoviridis; synonym; Straw itch. *Journal of the Indiana State Medical Association*, **2**, 337.

Rook, A., Ebling, F.J. & Wilkinson, D.S. (1968) *Textbook of Dermatology*, Vol. 1, p. 1007. Blackwell Scientific Publications, Oxford.

Savinetti, A. (1926) Contributo allo studio del 'pediculoides ventricosus' causa di una dermatite eritremopapulosa, manifestasi in forma epidemica tra gli scaricatori del porto di Trieste. *Archivio italiano di dermatologia, sifilografia e venereologia*, **4**, 224.

Schamberg, J.F. (1910) Grain itch. *Journal of Cutaneous (and Genito-Urinary) Diseases including Syphilis*, **28**, 67.

Sergent, E. & Alary, A. (1916) Petite épidémie d'acariose en Algerie. *Bulletin de la Sociéte de pathologie exotique*, **9**, 771.

Stelwagon, H.W. (1921) *Treatise on diseases of the Skin*, 9th edn, p. 1242. W.B.Saunders, Philadelphia and London.

Swan, D.C. (1934) The hay itch mite, *Pyemotes ventricosus* (Newport) (Acarina Pediculoididae) in South Australia. *Journal of Agriculture and Industry of South Australia*, **37**, 1289.

Thompson, A.G.G. (1925) Barley itch. *British Medical Journal*, **i**, 71.

Webster, F.M. (1910) A predaceous and supposedly beneficial mite, *Pediculoides* becomes noxious to man. *Annals of the Entomological Society of America*, **3**, 15.

Willcocks, F.C. (1914) The predaceous mite *Pediculoides ventricosus* Newport. *Agricultural Journal of Egypt*, **4**, 31.

Wills, W.K. (1909) Barley itch. *British Journal of Dermatology*, **21**, 249.

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Pilo-sebaceous duct physiology

2. THE EFFECT OF KERATIN HYDRATION ON SEBUM EXCRETION RATE

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SUMMARY

Sebum excretion rate was measured before and after occlusion of the skin in eleven subjects. The sebum excretion rate rose significantly following occlusion, confirming the view that there is an obstruction to the outflow of sebum with keratin hydration.

Obstruction of the pilo-sebaceous duct by accumulation of an excessive amount of keratin is an important factor in the pathogenesis of acne (Kligman, 1974). Using a surface microscope, Williams, Cunliffe & Gould (1974) showed that the pilo-sebaceous exits become smaller as a result of keratin hydration produced by a hot bath or polythene occlusion. They postulated that this decrease in exit size could cause an increased sebum outflow resistance, possibly explaining the exacerbation of acne seen premenstrually and in hot humid environments. We therefore thought that we should investigate the functional effect of skin hydration on sebum excretion rate.

MATERIALS AND METHODS

Eleven acne patients aged between 15 and 40 years and with a mean age of 26·0±3·1 years were investigated. These patients were not on any treatment locally or systemically at the time of the investigation or for 6 weeks before the test. Sebum was collected from the left and right sides of the upper back using the method of Strauss & Pochi (1961) modified by Cunliffe & Shuster (1969). Symmetrical areas were delineated on the back over the medial borders of the scapulae and the absorbent papers were held in place by an elasticated bandage fastened in front of the chest with nylon mesh. On the first day the sebum was collected at ½ h periods for 2 h from one side (the test side), and for a continuous period of 2 h from the other side (the control side). Sebum was collected in this way to control for the possibility that by using $4 \times \frac{1}{2}$ h collections excessive amounts of sebum could be obtained due to the papers acting as a wick.

Following this collection the test area on the back was occluded with polythene, which was held in place by 'Blenderm' surgical tape (Minnesota Mining and Manufacturing Co.). Twenty-two hours later the polythene was removed and the sebum collected at the same time intervals as on the previous day. All collections were made at the same time to control for any circadian changes in sebum excretion rate. The room temperature and the skin temperature at the site of collection were noted on both days.

RESULTS

Before sebum is collected for measurement of sebum excretion rate, preparatory papers are placed on the skin until the so-called follicular pattern is achieved, this usually takes 20–30 min. It was noted on the 2nd day on the test side that there was only a little lipid on the preparatory paper; the control side preparatory papers visually showed normal quantities. We carefully examined the preparatory papers on the test side for the presence of excessive moisture. Only slight moisture was seen and this would in no way interfere with the collection of sebum from the skin.

There was no significant difference between the sebum excretion rate on the control side and the test side on the 1st day and between the control side on the 1st day and the 2nd day (Fig. 1). On the 1st day the first $\frac{1}{2}$ h collection on the test side was higher than the subsequent ones, as expected (Cunliffe & Shuster, 1969), but the mean rate over the 2 h was not significantly different from the other side. On the 2nd day the total amount of sebum collected was almost 3 times more on the test side than on the control side, and analysis of the $\frac{1}{2}$ h collections showed that for the first $\frac{1}{2}$ h the rate on the test side was similar to that on the previous day. Thereafter on the test side the sebum excretion rate rose significantly, reaching a maximum at 90 min and then returning almost to the expected rate at 120 min (Fig. 2). There was no significant difference between the room and the skin temperature on the 1st day and the 2nd day.

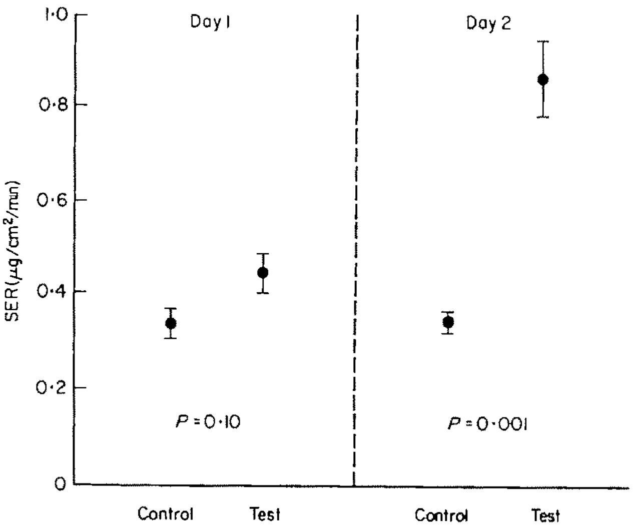

FIGURE 1. The effect of skin hydration on sebum excretion rate using 2 h collections.

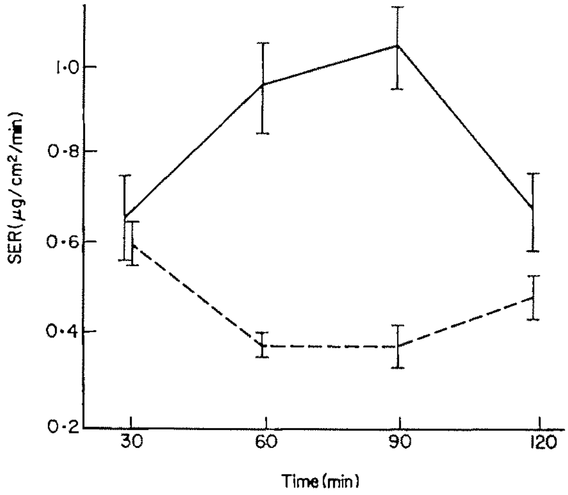

FIGURE 2. Sebum excretion rate at ½ h intervals after removal of occlusion. ——, Test; - - - -, control. N = 11.

DISCUSSION

It was impossible to collect sebum while the polythene was in place and so we were unable to measure the sebum excretion rate during occlusion. The purpose of the preparatory papers in this method of measuring sebum excretion is to remove from the skin surface excess moisture and sebum already present on the skin, so assuring that the lipids absorbed by the final papers truly represent an excretion rate. Therefore, precise measurements could not be made before a follicular pattern had been obtained. The apparent reduction in lipid absorbed by the preparatory papers was an observation made in all eleven patients.

This could represent the inability of the preparatory papers to pick up the lipid because of excess moisture; none of the papers became crinkled and they showed only minimal sweat, certainly insufficient to affect the pick-up of sebum. Therefore, there is a real difference in the amount of surface lipid collected by the preparatory papers on the test and control sides. The sebum excretion rate gradually rose following removal of polythene to 2–3 times the rate on the control side. There are several possible explanations for such an observation.

A rise of 1°C increases sebum excretion rate by 10% (Cunliffe, Burton & Shuster, 1970) and so an increase in sebum excretion rate after polythene occlusion could be explained on the basis of increased temperature under the occlusion. However, we did not find any significant increase in temperature on the test side immediately after removal of the occlusion or in the following 2 h. No individual showed a difference of more than 0·8°C which would not be sufficient to explain the result of the sebum excretion rate.

Increased activity of the gland would be another explanation for an observed sebum excretion rate but the short time dependency of the results indicates that it is more likely to be due to a local phenomenon. It has already been shown that keratin hydration reduces the pilo-sebaceous duct exit size and that the duct returns to its original size in 90 min (Williams *et al.*, 1974). The present findings can be explained on the basis that sebum excretion rate was reduced during the occlusion when the ducts became smaller. This would lead to a build up of sebum within the pilo-sebaceous duct and a rise in sebum excretion rate when the duct returns to its original size.

From the results it is possible to calculate the extent of the obstruction, at least in terms of sebum outflow. If we assume that the rate of sebum excretion over the 22 h (whilst the polythene is in place) is constant at about 0·4 $\mu g/cm^4/min$ then in 22 h $0{\cdot}4 \times 60 \times 22 = 528$ $\mu g/cm^4$ would have been produced. This is an approximation as it is known that sebum excretion rate shows a diurnal variation (Burton, Cunliffe & Shuster, 1970). On this basis it would be expected that 48 μg of sebum would be collected in a 2 h period. On the test side after removal of the polythene the actual amount collected was about 96 μg, i.e. 48 μg in excess of the expected value. This is only a small fraction (8%) of the amount excreted on the unoccluded back over 22 h showing that the effective obstruction to sebum outflow is small.

However, we have measured sebum excretion from many sebaceous follicles and there is evidence (Kligman, 1974) that in an acne-prone skin some follicles are at the stage of developing micro-comedones. It is therefore reasonable to suggest that these are more easily blocked than a normal sebaceous follicle and in these structures the damming back of sebum could have profound effects in accelerating the development of acne lesions.

Therefore, the observed results are best explained by their correlation with the behaviour of a pilo-sebaceous duct exit site after polythene occlusion. Sebum cannot be collected during polythene occlusion and so our evidence is only indirect. Nevertheless, our data suggest that hydration of keratin results in an increased resistance to sebum outflow, causing a slight build up of sebum which escapes as the duct returns to its normal size.

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REFERENCES

BURTON, J.L., CUNLIFFE, W.J. & SHUSTER, S. (1970) Circadian rhythm in sebum excretion. *British Journal of Dermatology*, **82**, 497.

CUNLIFFE, W.J., BURTON, J.L. & SHUSTER, S. (1970) The effect of local temperature variations on the sebum excretion rate. *British Journal of Dermatology*, **83**, 650.

CUNLIFFE, W.J. & SHUSTER, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 650.

KLIGMAN, A.M. (1974) An overview of acne. *Journal of Investigative Dermatology*, **62**, 268.

STRAUSS, J.S. & POCHI, P.E. (1961) The quantitative gravimetric determination of sebum production. *Journal of Investigative Dermatology*, **36**, 293.

WILLIAMS, M., CUNLIFFE, W.J. & GOULD, D. (1974) Pilo-sebaceous duct physiology. 1. *British Journal of Dermatology*, **90**, 631.

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Mycobacterium marinum skin infections mimicking cutaneous leishmaniasis

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SUMMARY

Ten cases of skin infection due to *Mycobacterium marinum* are reported from Israel. Most of the infections were contracted in natural bathing pools at Ein Feshka on the shores of the Dead Sea, south of Jericho. The lesions closely resembled those of cutaneous leishmaniasis prevalent in this region. The condition does not seem to have been detected previously in the Middle East.

Skin granulomas due to *Mycobacterium marinum* (balnei) have been recognized since the early 1950s following reports by Linell & Nordén (1954) and Herlitz (1953) on 80 cases infected at one swimming pool. Most earlier instances of 'swimming-pool granuloma' (Hellerström, 1952) were probably due to the same organism. Four epidemics account for 523 of the more than 600 cases so far published: these occurred in Sweden, the U.S.A. and Britain (Linell & Nordén, 1954; Zettergren & Zetterberg, 1956; Philpott *et al.*, 1963; Waddington, 1967). Sporadic cases or small outbreaks have been reported from Canada, Holland, Belgium, Czechoslovakia, Australia, New Zealand and Japan. The detection of *M. marinum* infection in the Middle East is of interest because the lesions mimic those of cutaneous leishmaniasis endemic in the same area.

CASE REPORTS

Three types of lesion were seen (Figs 1, 2). A general description follows and additional clinical data for each case are presented in Table 1. The first type of lesion, present in all cases, consisted of chronic inflammatory nodules or plaques 1–4 cm in diameter, with scales, crusts or small ulcers on their surface. Beneath the crusts was a slight sero-sanguinous or purulent discharge. The surface of the plaques was uneven, occasionally with a smooth atrophic area. Small satellite papules were present in most cases and constituted the second type of lesion; they usually had a central scale, crust or ulcer. The third type of lesion was seen in four of the ten patients and consisted of one or more subcutaneous or intradermal cyst-like swellings. These were 1·5–2·0 cm in diameter, some distance proximal to the original lesions, and if multiple were arranged along an axis in sporotrichoid fashion.

The condition began as one or more small papules which rapidly became swollen and discharged pus. The discharge became scanty or dried and the lesion enlarged. Satellite papules and sporotrichoid

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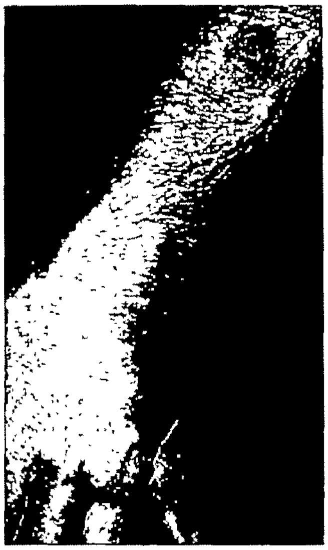

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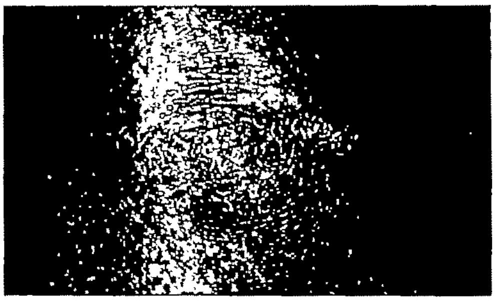

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FIGURE 1. Case 2. The nodular, ulcerated lesion on the hand appeared 2 weeks after a period of bathing at Ein Feshka. Four months later a smooth, cystic, sporotrichoid swelling developed 13 cm proximally; when incised this released a gelatinous material and subsequently ulcerated. *M. marinum* was isolated from both lesions.

FIGURE 2. Case 3. The larger, irregular, nodular plaque with scales, small crusted ulcers and partial central atrophy developed on an unhealed graze of the right knee which occurred while bathing at Ein Feshka. The smaller lesion appeared 8 months later without further bathing. *M. marinum* was isolated from both lesions.

lesions appeared later, at intervals which varied up to 18 months. The lesions were usually asymptomatic, the regional lymph nodes were not enlarged and there was no disturbance of the general health.

The clinical picture was similar to that of cutaneous leishmaniasis, locally called 'Jericho boil', and all but two of the patients had visited the Jericho area. When smears and cultures for *Leishmania tropica* and the intradermal leishmanin tests proved negative routine, bacteriological studies were done and a search made for mycobacteria. Biopsies were done in eight of the cases.

Bacteriological studies

Tissue scraped from incisions, biopsy material, exudates, crusts and scales, was examined. The specimens were ground aseptically, washed once in normal saline and suspended in phosphate buffer, pH 7·2. Specimens showing no growth on blood agar plates were cultured on Loewenstein–Jensen (L–J) medium without further treatment. Specimens showing growth were treated for 15 min with 6% sulphuric acid, washed with saline, resuspended in phosphate buffer and inoculated on L–J medium. The cultures were incubated at 32°C and 37°C.

On microscopic examination of smears a few, scattered, acid-fast bacilli were seen in specimens from five out of the ten cases. In cultures from all ten cases, small creamy-white colonies which became yellow–orange after exposure to light appeared after 7–12 days at 32°C. There was no growth at 37°C. The cultures were identified as *M. marinum* by their photochromogenecity, rate of growth and need for lower temperature. Identification was confirmed by hydrolysis of Tween 80, negative nitrate reductase and positive arylsulphatase activity.

TABLE 1. Clinical data on ten cases of *M. marinum* skin infection

Patient	Sex	Age	Sites affected	Trauma	Water exposure	Sporotrichoid lesions	Incub. period	Treatment and course
1	M	40	Elbow, upper arm	—	Ein Feshka	Yes	2 wk–2 mth.	None: complete resolution
2	M	9	Hand, forearm	—	Ein Feshka Galilee stream Medit'n	Yes	2 wk–2 mth.	Grenz rays: partial resolution
3	F	9	Knee	Graze (while bathing)	Ein Feshka	—	Brief	Grenz rays: partial resolution
4	M	39	Elbow, upper arm	Graze (while bathing)	Ein Feshka Medit'n	Yes	Brief	Grenz rays: complete resolution
5	M	21	Knee, thigh	—	Sea of Galilee	Yes	?	Griseofulvin: complete resolution
6	F	47	Forearm, hand	Graze (from gas range)	—	—	Brief	Grenz rays: course not known
7	F	7	Knee	Kick (before bathing)	Ein Feshka	—	6 mth.	Grenz rays: partial resolution
	M	61	Upper arm	Insect bite (before bathing)	Ein Feshka	—	Brief	None: complete resolution
9	M	12	Buttock	Insect bite (while bathing)	Ein Feshka	—	1–2 mth.	Grenz rays: partial resolution
10	F	38	Forearm	—	Ein Feshka	—	2 yr.	None: course not known

Drug sensitivity tests were done on L–J medium containing various dilutions of isoniazid, PAS, streptomycin, ethambutol and rifampicin. Disc susceptibility tests were performed on Dubos oleic agar base, using cotrimoxazole (25 μg) and vancomycin (5 μg) discs (Baltimore Biological Laboratories, U.S.A.). The results are given in Table 2.

Purified protein derivative (PPD) tests

The results of intradermal tests with PPD from 6 various sources are given in Table 3.

Histopathology

Marked changes were observed in seven of the eight patients from whom biopsy specimens were taken (Fig. 3). The epidermis showed hyperkeratosis with focal parakeratosis, irregular acanthosis with pseudoepitheliomatous hyperplasia or extreme elongation of the rete pegs, and liquefaction degeneration of the basal layer. In the upper- and mid-dermis there was a mixed inflammatory infiltrate which sometimes extended into the epidermis. The cells were mainly lymphocytes and histiocytes but in two patients plasma cells predominated. There was a marked neutrophilic and eosinophilic exudate in two cases and in six cases microabscesses were present, usually located near

TABLE 2. Sensitivity of nine *M. marinum* strains to antibacterial agents (compared with a standard strain of *M. tuberculosis*)

Strain from patient no.	Minimum inhibitory concentration (μg/ml)					Disc sensitivity test	
	Isoniazid	PAS	Streptomycin	Ethambutol	Rifampicin	Cotrimoxazole (25 μg)	Vancomycin (5 μg)
1	>5	>100	10	<1	10	R*	R*
2	>5	>100	10	4	>10	R	R
3	5	>100	10	2	10	R	R
4	5	>100	10	2	10	R	R
5	>5	>100	10	2	10	R	R
6	5	>100	10	1	10	R	R
7	>5	>100	10	2	10	R	R
8	5	>100	10	2	10	R	R
9	5	>100	10	2	10	R	R
M. tuberculosis (H37RV)	0·2	1	1	2	5	—	—

* Resistant (no inhibitory zone around discs).

the tip of an elongated rete peg or in a dermal papilla. Non-caseating epithelioid granulomas were seen in all seven cases and in one instance the epithelioid granulomas were invaded by neutrophils. In most cases multinucleated giant cells of Langhans' or foreign body type were present (Fig. 4a, b). In the eighth patient there was a mixed histiocytic and lymphocytic infiltrate, with a few neutrophils, around the deep dermal vessels: no granulomas, giant cells or microabscesses were seen. Acid-fast bacilli were found in one case. Leishman-Donovan bodies and fungi could not be demonstrated.

Treatment and course

Five patients were treated twice with 500–700 rad Grenz rays 3–26 months after the onset of their condition; the interval between the radiations in the different cases varied from 3 weeks to 4 months (Table 1: patients nos. 2, 3, 4, 7, 9). Complete resolution occurred within 2 months in patient no. 4 and moderate to marked resolution in the others, who are still under observation. Patient no. 6 received 700 rad Grenz rays once to the lesions but did not report for further examination. Complete resolution occurred in three patients without specifically directed treatment. In patient no. 8 this occurred 4 months after the lesions appeared, bacitracin ointment only being applied. The elbow

TABLE 3. Results of intradermal tests with five units PPD from various sources (mm induration after 72 h)

Source of PPD	Patient									
	1	2	3	4	5	6	7	8	9	10
M. marinum	12	7	7	8	12	21	19	0	6	0
M. tuberculosis	13	0	5	8	10	20	16	6	5	0
M. kansasii	17	12	6	0	15	24	14	7	6	0
M. fortuitum	0	5	0	5	0	0	0	2	0	0
Scotochromogens	9	8	13	15	16	16	7	0	0	5
Battey	13	7	12	10	12	18	5	5	4	9

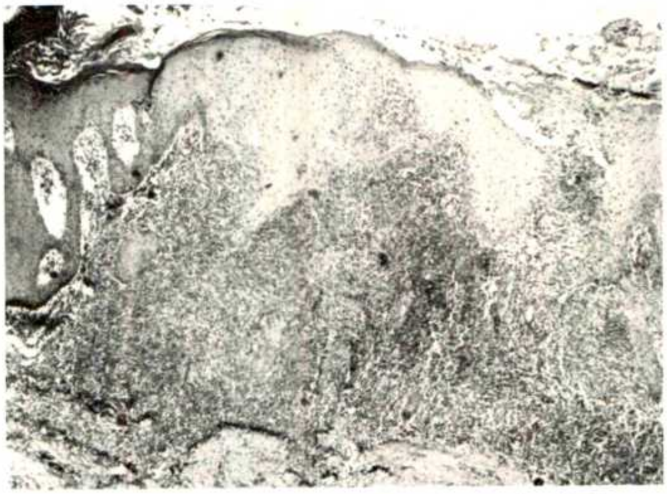

FIGURE 3. Typical lesion with hyper- and parakeratosis, pseudoepitheliomatous hyperplasia and a dense inflammatory infiltrate in the dermis (H & E, × 42).

lesion in patient no. 1 had been diagnosed elsewhere as psoriasis and had been treated by 4 intralesional injections of triamcinolone acetonide. After initial marked improvement the lesion became swollen, discharged pus and 2 months later a sporotrichoid swelling appeared on the upper arm. After a further 8 months complete regression occurred without other treatment. Patient no. 5 had been given fine-particle griseofulvin by another doctor shortly before the diagnosis was made. This treatment was continued and 3 months later there was resolution of the lesions. The last patient (no. 10) did not report after the initial visit at which material was taken for examination.

DISCUSSION

Mycobacterium marinum is capable of living saprophytically, especially in an aquatic environment, and parasitically in cold-blooded animals and man. The fact that in man the lesions are nearly always

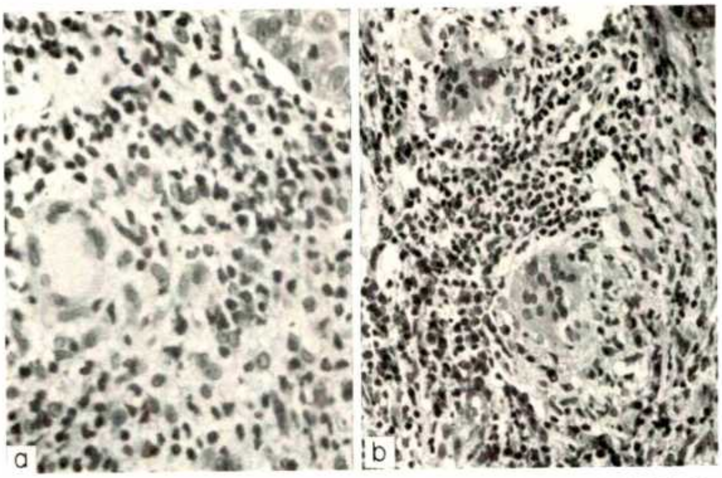

FIGURE 4. (a) Multinucleated giant cell of the Langhans type (H & E, × 420). (b) Multinucleated giant cell of the foreign body type (H & E, × 260).

limited to the skin has been attributed to the preference of the parasite for relatively cool growth conditions. Only occasionally has involvement of deeper structures been demonstrated or suspected (Winter & Runyon, 1965; Gould, McMeekin & Bright, 1968; Jolly & Seabury, 1972; Cortez & Pankey, 1973). The lesions are usually on one limb and few in number but widespread skin infection has occurred in patients with suspected immunological impairment (Gould *et al.*, 1968; Sage & Derrington, 1973).

Human infection is usually acquired whilst bathing in fresh, brackish or salt water. However, 'swimming-pool granuloma' is not a suitable synonym, for lakes, wells, rivers, inlets, bays, the seashore, home aquaria and a coal mine have been incriminated. Some patients have denied contact with any extra-domiciliary source of water. Previous trauma of the affected site, although common, is not always recalled.

Eight of the ten patients in this series were apparently infected at Ein Feshka, a bathing area south of Jericho where fresh-water pools fed by springs empty into the Dead Sea via short canals. By coincidence, this region is highly endemic for cutaneous leishmaniasis, the lesions of which may be clinically indistinguishable from those due to *M. marinum*. This coincidence has probably delayed recognition of the latter condition. The incubation period was very variable. Although most commonly a few weeks to a few months, in one case it may have been as long as 2 years whilst in others it seemed virtually absent, the lesions rapidly developing on a traumatized site which did not heal. It was not possible to assess the incubation period in those cases without a history of trauma and with no limited exposure period to a suspected water-source.

The examination of multiple samples is important for the detection of *M. marinum* in the lesions. Acid-fast bacilli are seen only rarely in histological sections and infrequently in smears, whilst cultures may not always be positive. In the present series, three to eight samples were taken from each patient. Of the total of forty-six samples, acid-fast bacilli were seen microscopically in six and there were only twenty-six positive cultures.

M. marinum is classified in Runyon's Group I together with *M. kansasii*, since both are photochromogenic. *M. kansasii* grows at 37°C whilst *M. marinum* grows at 30–32°C. However, primary isolation of *M. marinum* at 37°C has been reported (Feldman, Long & David, 1974): confirmation of identity may be made by the nitrate reduction test, usually negative for *M. marinum* and positive for *M. kansasii* (Silcox & David, 1971). *M. ulcerans* also requires the lower incubation temperature but its growth takes several weeks. Skin lesions due to *M. ulcerans* possess an abundance of acid-fast bacilli and on culture the colonies are non-pigmented, tend to form cords and give a positive neutral red test.

The histopathological picture is usually suggestive but not pathognomonic. The diagnosis is supported by the presence of a mixed inflammatory reaction with a dense mononuclear infiltrate, a few non-caseating granulomas with or without giant cells, and microabscesses. Central fibrinoid necrosis in the granulomas has been reported occasionally and caseating necrosis rarely (Linell & Nordén, 1954; Schaefer & Davis, 1961). Acid-fast bacilli are not usually seen and their detection requires a prolonged search of serial sections.

Clinical and histological differential diagnosis includes all other chronic infective granulomas, foreign body granuloma and iodine and bromine granulomas. Clinically, in addition, psoriasis, verrucous lichen planus and even verruca vulgaris may have to be considered. In the Middle East confusion is most likely with cutaneous leishmaniasis and some features differentiating the two conditions are given in Table 4. A positive reaction of 10 mm or more induration to the intradermal PPD (marinum) test supports the diagnosis but the result must be interpreted with caution. There is a high incidence of cross-reactivity with PPD from other sources and in the patients in this series the reaction to PPD (marinum) was not usually the strongest (Table 3).

TABLE 4. Differential diagnosis of *M. marinum* and *L. tropica* skin infection

	Mycobacterium marinum infection	*Leishmania tropica* infection
Patient's history		
Geographic factors	Not known: reported so far from only a few widely dispersed areas	Sub-tropical and tropical endemic areas
Seasonal factors	Probably not relevant, but if infected whilst bathing outdoors usually during warmer season	During warmer season
Diurnal factors	Probably not relevant, but if infected whilst bathing outdoors usually during daylight hours	Acquired at or after sunset
Exposure to water source	Commonly reported	Not relevant
Minor trauma	Commonly reported	None, except insect bite
Incubation period	Very brief to prolonged	Usually not less than 4 weeks. May be prolonged
Clinical appearance		
Number and site of lesions	Few, usually on limb at site liable to trauma: rarely bilateral	Few or numerous. Common on face as well as limbs: often bilateral
Sporotrichoid lesions	Relatively common	Rare
Special examinations		
Intradermal tests*	PPD tests .... positive Leishmanin test .... negative	PPD tests .... negative Leishmanin test .... positive
Smears	Acid-fast bacilli found with difficulty	Leishman–Donovan (LD) bodies found easily in early lesions
Culture	From tissues and discharge: *M. marinum* may not grow from all the specimens	From tissue at edge of lesion: *L. tropica* usually grows from all early lesions
Histologic picture	Acute and chronic inflammatory infiltrate. May be giant cells, epithelioid granulomas and microabscesses. A–F bacilli rare	Chronic inflammatory infiltrate sometimes granulomatous. Numerous LD bodies in histiocytes in early lesions

* In areas in which both leishmanial and mycobacterial infections occur the intradermal tests must be interpreted with care for positive reactions persist after cure.

Spontaneous healing probably occurs within a few months to a few years in the majority of cases. Small, rapidly healing lesions may never be brought to the attention of a doctor: such cases, if frequent, may be responsible for an increased percentage of tuberculin-positive reactors in some areas (Judson & Feldman, 1974).

The many treatments recommended include excision with skin grafting, curettage, electrodesiccation or fulguration, cryotherapy, local heat, ultraviolet irradiation, Grenz-ray therapy, X-ray therapy, intralesional injection of corticosteroids, potassium iodide, vaccine therapy, chemotherapy and the use of antibiotic and antituberculous drugs. In refractory cases a combination of treatment is indicated, possibly combined with oral corticosteroids.

Drug therapy should be preceded by sensitivity studies, for the response of different strains may vary. All the nine strains examined in the present series were sensitive to ethambutol and partially sensitive to streptomycin and rifampicin: they were resistant to isoniazid, PAS, cotrimoxazole and vancomycin (Table 2). Strains sensitive to cotrimoxazole and vancomycin have been reported by others (Flowers, 1970; Adams *et al.*, 1970). Resistance to isoniazid and PAS, uniformly reported for *M. marinum*, is highly characteristic of all atypical mycobacteria.

Epidemiological studies are under way at Ein Feshka. Samples of water from the pools, canals and shower-room facilities, earth and stones from the bottom of the pools, and scrapings from rocks at the pool edges have been examined. Most of the specimens were highly contaminated with other micro-organisms. Acid-fast bacilli were seen microscopically in three out of sixteen specimens and *M. marinum* was isolated from two out of fifty-four cultures. The studies will be reported on in detail at a later date and it is hoped that on their completion means may be devised to eradicate the source of infection.

ACKNOWLEDGMENTS

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REFERENCES

ADAMS, R.A., REMINGTON, J.S., STEINBERG, J. & SEIBERT, J.S. (1970) Tropical fish aquariums: a source of *Mycobacterium marinum* infection resembling sporotrichosis. *Journal of the American Medical Association*, **211**, 457.

CORTEZ, L.M. & PANKEY, G.A. (1973) *Mycobacterium marinum* infections of the hand: Report of three cases and review of the literature. *Journal of Bone and Joint Surgery*, **55**, 363.

FELDMAN, R.A., LONG, M.W. & DAVID, H.L. (1974) *Mycobacterium marinum*: a leisure-time pathogen. *Journal of Infectious Diseases*, **129**, 618.

FLOWERS, D.J. (1970) Human infection due to *Mycobacterium marinum* after a dolphin bite. *Journal of Clinical Pathology*, **23**, 475.

GOULD, W.M., MCMEEKIN, D.R. & BRIGHT, R.D. (1968) *Mycobacterium marinum* (balnei) infection: report of a case with cutaneous and laryngeal lesions. *Archives of Dermatology*, **97**, 159.

HELLERSTRÖM, S. (1952) Water-borne tuberculous and similar infections of the skin in swimming pools. *Acta dermato-venereologica*, **32**, 449.

HERLITZ, S. (1953) Cutaneous infection contracted in a swimming pool and resembling tuberculosis. *Acta dermato-venereologica*, **33**, 156.

JOLLY, H.W. JR. & SEABURY, J.H. (1972) Infections with *Mycobacterium marinum*. *Archives of Dermatology*, **106**, 32.

JUDSON, F.N. & FELDMAN, R.A. (1974) Mycobacterial skin tests in humans 12 years after infection with *Mycobacterium marinum*. *American Review of Respiratory Diseases*, **109**, 544.

LINELL, F. & NORDÉN, A. (1954) *Mycobacterium balnei*: a new acid-fast bacillus occurring in swimming pools and capable of producing skin lesions in humans. *Acta Tuberculosea et Pneumologica Scandinavica*, Supplement 33.

PHILPOTT, J.A. JR., WOODBURNE, A.R., PHILPOTT, O.S., SCHAEFER, W.B. & MOLLOHAN, C.S. (1963) Swimming pool granuloma: a study of 290 cases. *Archives of Dermatology*, **88**, 158.

SAGE, R.E. & DERRINGTON, A.W. (1973) Opportunistic *Mycobacterium marinum* infection mimicking *Mycobacterium ulcerans* in lymphosarcoma. *Medical Journal of Australia*, **2**, 437.

SCHAEFER, W.B. & DAVIS, C.L. (1961) A bacteriologic and histologic study of skin granuloma due to *Mycobacterium balnei*. *American Review of Respiratory Diseases*, **84**, 837.

SILCOX, V.A. & DAVID, H.L. (1971) Differential identification of *Mycobacterium kansasii* and *Mycobacterium marinum*. *Applied Microbiology*, **21**, 327.

WADDINGTON, E. (1967) An outbreak of swimming pool granuloma. *Transactions and Report of the St John's Hospital Dermatological Society*, **53**, 122.

WINTER, F.E. & RUNYON, E.H. (1965) Prepatellar bursitis caused by *Mycobacterium marinum* (balnei). *Journal of Bone and Joint Surgery*, **47**, 375.

ZETTERGREN, L. & ZETTERBERG, B. (1956) The swimming pool disease: *Mycobacteriosis balnearea*. *Acta Societatis Medicorum Upsaliensis*, **61**, 47.

British Journal of Dermatology (1976) **94**, 443.

Lysosomal hydrolases of the epidermis

4. OVERALL PROFILE IN COMPARISON WITH DERMIS AND OTHER TISSUES

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SUMMARY

The activities of fifteen acid hydrolases have been measured in seven tissues of the guinea-pig; fourteen of these were also assayed in the epidermis of four other mammalian species. The most striking finding was that the proportion of acid phosphatase was consistently much higher in epidermis than in the other tissues investigated.

The function of lysosomes is, in a general sense, concerned with the degradation of biological macromolecules. Nevertheless, since there are wide differences in the turnover of macromolecules by the different tissues, one might expect to find corresponding variations in their patterns of hydrolase activity.

Values for the acid hydrolase activities of most tissues, including epidermis, are now available. Unfortunately comparison of these figures is exceedingly hazardous, since different investigators have used different extraction techniques, selected different substrates and other reaction conditions, and have expressed the activities in terms of various reference variables (per unit fresh weight, dry weight, soluble protein, total protein, etc.). In addition significant species variation has been reported.

We have therefore employed the fluorimetric assay techniques described previously (Mier & v.d.Hurk, 1975a–c) to obtain a direct comparison between the acid hydrolase profiles of epidermis and those of other tissues in the guinea-pig; we have also extended our study of epidermis to include a representative selection of mammalian species. Our findings are reported below.

MATERIALS AND METHODS

Albino guinea-pigs (6–8 weeks) were killed by a blow to the head. Samples of tissue (Table 1) were quickly removed and stored in liquid nitrogen prior to analysis. Epidermis was collected from all animals (Table 2) and from human subjects using a keratotome set for a depth of 0·2 mm. Homogenization was carried out in a solution of bovine serum albumin (1 mg/ml) using a 'Micro-wet grinder' (Townson and Mercer) fitted with an ice-jacket. The homogenates were centrifuged to yield clear supernatants and dilutions prepared with bovine serum albumin solution.

TABLE 1. Mean activities of fifteen acid hydrolases in each of seven tissues of the guinea-pig

EC no.	Enzyme	Liver (2)	Kidney (2)	Spleen (1)	Intestine (2)	Brain (2)	Dermis (2)	Epidermis (7)
3.1.3.2	Acid phosphatase	1800	1400	3600	840	310	97	1100
3.1.4.1	Pyrophosphatase	94	53	170	24	5.7	0.81	56
3.1.6.1	Arylsulphatase A	3·0	6·1	5·0	2·2	1·1	0·057	0·46
3.1.6.2	Arylsulphatase B	6·2	11	7·6	6·2	4·7	0·11	1·3
3.2.1.20	α-glucosidase	360	240	170	360	75	2·1	5·3
3.2.1.21	β-glucosidase	260	17	51	410	8·5	1·2	3·5
3.2.1.22	α-galactosidase	100	110	270	99	44	1·6	7·8
3.2.1.23	β-galactosidase	400	260	540	240	77	18	38
3.2.1.24	α-mannosidase	170	84	99	52	19	2·3	7·8
3.2.1.30	β-acetylglucosaminidase	5000	2700	6200	2200	810	36	25
3.2.1.31	β-glucuronidase	2900	350	2400	700	83	8·7	8·1
3.4.4.-	Cathepsin B_1	350	210	450	820	35	34	22
3.4.4.9	Cathepsin C	4900	960	8900	2000	72	58	42
3.4.4.23	Cathepsin D	10	5·8	8·9	4·9	2·8	0·68	0·86
3.5.1.-	Arylamidase	2800	15000	2900	2700	2000	27	120

All values are in nmol/min/g wet wt tissue.

Figures in parentheses indicate the numbers of different animals investigated; in the case of epidermis, this includes four values published previously (Mier & v.d.Hurk, 1975a–c).

TABLE 2. Mean activities of fourteen acid hydrolases in the epidermis of various mammalian species

EC no.	Enzyme	Guinea-pig (7)	Monkey (2)	Cow (2)	Rabbit (2)	Human (27)
3.1.3.2	Acid phosphatase	1100	140	5700	500	830
3.1.6.1	Arylsulphatase A	0·46	3·1	0·21	0·06	0·44
3.1.6.1	Arylsulphatase B	1·3	1·2	1·0	0·07	0·40
3.2.1.20	α-glucosidase	5·3	5·7	10	2·9	10
3.2.1.21	β-glucosidase	3·5	0·61	4·9	3·3	1·3
3.2.1.22	α-galactosidase	7·8	4·1	5·6	7·1	9·8
3.2.1.23	β-galactosidase	38	27	15	63	37
3.2.1.24	α-mannosidase	7·8	6·9	93	6·6	29
3.2.1.30	β-acetylglucosaminidase	25	46	300	29	130
3.2.1.31	β-glucuronidase	8·1	28	17	11	41
3.4.4.-	Cathepsin B_1	22	2·7	1·3	5·2	5·8
3.4.4.9	Cathepsin C	42	17	26	11	62
3.4.4.23	Cathepsin D	0·86	2·6	3·4	2·0	1·7
3.5.1.-	Arylamidase	120	160	520	140	280

Values are in nmol/min/g wet wt tissue.

Enzyme activities were measured using duplicate 0·1 ml samples of the tissue extracts. Except for the concentrations of the extracts (the optimum values of which were determined by preliminary experiments), the assay methods were in all respects exactly as described previously (Mier & v.d. Hurk, 1975a–c).

RESULTS

The activities of fifteen hydrolases in seven different tissues of the guinea-pig are shown in Table 1, and the activities of fourteen hydrolases from the epidermis of five mammalian species are compared in Table 2.

DISCUSSION

Despite the enormous range of activities (more than 5 orders of magnitude) seen in Table 1, certain patterns emerge. First, the 'profiles' of hydrolase activity of most tissues, although by no means identical, tend to be relatively constant. Secondly, the absolute values are generally much higher for those tissues whose function involves the degradation of large quantities of exogenous macromolecules (liver, kidney, etc.) than those tissues which do not serve such a role (brain, connective tissue, epidermis). Neither of these findings is especially remarkable, being implicit in the lysosome concept as outlined by De Duve & Wattiaux (1966).

Particular importance must therefore be attached to the one exception to this generalization, namely the extremely high level of phosphate esterase activity found in epidermis. This is better illustrated in Table 3, where the contribution of each group of enzymes has been calculated as a percentage of the total hydrolase activity. Expressed in this way, both the phosphate mono- and diesterases are an order of magnitude higher in epidermis than in any other tissue except dermis. It is possible that this last figure reflects contamination with a small percentage of keratinocytes originating from the hair follicles.

TABLE 3. Percentage contribution of each group of enzymes to the total hydrolase activity for the various tissues

Enzyme group	Liver	Kidney	Spleen	Intestine	Brain	Dermis	Epidermis
Acid phosphatase	9·4	6·4	14	8·0	8·6	34	76
Pyrophosphatase	0·49	0·24	0·66	0·23	0·16	0·29	3·9
Sulphatases	0·05	0·08	0·05	0·08	0·16	0·06	0·12
Glycoside hydrolases	48	17	38	39	31	24	6·6
Peptide hydrolases	42	76	48	53	60	42	13

Table 2 confirms earlier reports (e.g. Jansén & Hopsu-Havu, 1969) indicating that real differences exist between the acid hydrolase distributions in the skin of different species. Nevertheless, the high proportion of acid phosphatase remains a constant and general feature, the proportion of this enzyme varying between 31% (monkey) and 85% (cow snout). It is difficult to avoid the conclusion that the phosphate esterases of epidermis serve some function unique to this tissue; further evidence regarding this point is presented in the following paper (Mier *et al.*, 1975).

REFERENCES

De Duve, C. & Wattiaux, R. (1966) Functions of lysosomes. *Annual Reviews of Physiology*, **28**, 435.

Jansén, C.T. & Hopsu-Havu, V.K. (1969) Proteolytic enzymes in the skin. II. A comparative study of skin homogenates of five mammalian species. *Acta dermato-venereologica*, **49**, 468.

Mier, P.D. & van den Hurk, J.J.M.A. (1975a) Lysosomal hydrolases of the epidermis. 1. Glycosidases. *British Journal of Dermatology*, **93**, 1.

MIER, P.D. & VAN DEN HURK, J.J.M.A. (1975b) Lysosomal hydrolases of the epidermis. 2. Ester hydrolases. *British Journal of Dermatology*, **93**, 391.

MIER, P.D. & VAN DEN HURK, J.J.M.A. (1975c) Lysosomal hydrolases of the epidermis. 3. Peptide hydrolases. *British Journal of Dermatology*, **93**, 509.

MIER, P.D., COTTON, D.W.K., VAN DEN HURK, J.J.M.A. & JONCKHEER-VENNESTE, M.M.H. (1975) Lysosomal hydrolases of the epidermis. 5. Variation with depth in the cow snout. *British Journal of Dermatology*, in press.

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Case Reports

Ocular cicatricial pemphigoid associated with practolol therapy

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SUMMARY

The clinical and laboratory findings in a case of serious cicatricial blistering of the ocular mucous membrane were comparable with those found in cicatricial pemphigoid (ocular mucous membrane pemphigoid).

Various complications have been reported during the use of practolol (Eraldin) (Rowland & Stevenson, 1972; Raftery & Denman, 1973; Hunt & Frew, 1975). Felix, Ive & Dahl (1974) were the first to report psoriasiform skin reactions due to practolol in fourteen patients. In six of eight patients examined the direct immunofluorescence test (DIF) revealed IgG and IgM in addition to complement in a fine granular pattern along the dermal–epidermal junction, resembling the DIF pattern found in lupus erythematosus.

Wright (1974, 1975) reported various eye signs due to practolol in twenty-seven cases, but mentioned that the features in his cases were unlike the conjunctival scarring seen in association with cicatricial pemphigoid.

Here we discuss the classification of an ocular mucosal syndrome due to long term usage of practolol.

CASE REPORT

A man aged 46 years had been treated since August 1971 for angina with practolol tablets (600 mg daily). In February 1974, 2½ years later, he developed a bilateral conjunctivitis, which did not improve in the following months in spite of various local therapies. In October 1974 the eye symptoms got worse. Small papules or vesicles were seen in infiltrated erythematous areas on the conjuctivae and scarring and shrinkage were noted symmetrically, but most strikingly at the site of the left upper palpebral conjunctiva (Fig. 1). At that time the patient showed also a psoriasiform rash localized on his chest, elbows, and on the sides of some digits.

The administration of practolol was stopped. Five weeks later the skin had recovered completely

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This case report was read before the Meeting of the Dutch Association of Dermatology, Utrecht, 15 February 1975.

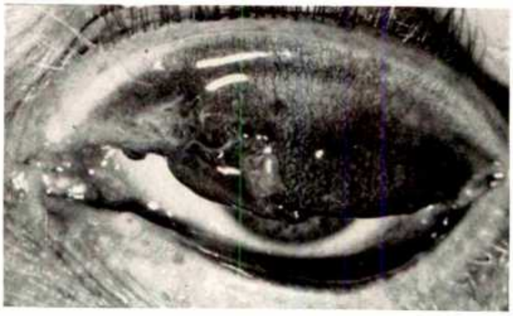

FIGURE 1. Left upper palpebral conjunctiva. Severe shrinkage, considerable scarring, and diffuse inflammation are visible. Note the small vesicles on the affected conjunctiva.

and no new lesions could be found on the conjunctivae although the old cicatricial lesions were still visible. Intracutaneous and epicutaneous skin tests to practolol were negative.

LABORATORY INVESTIGATIONS

The routine laboratory examinations, performed shortly before the administration of practolol was stopped, gave normal values. LE-cells (class 4) were, however, found in the patient's blood and antinuclear antibodies (ANA) were detectable in a titre of 1:80. Assays for anti-DNA antibodies were negative. One month after the practolol had been stopped the LE-cell phenomenon was dubiously (class 3) positive and ANA were only weakly positive. After another month, the assay for ANA was still weakly positive, but LE-cells were no longer demonstrable (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service).

Histopathological examinations:

(i) Lesional ocular mucous membrane showed subepithelial cleavage at the basement membrane zone. The submucosa showed an infiltrate composed largely of lymphocytes with plasmacytes and also some eosinophils and a great number of dilated blood vessels.

(ii) A psoriasiform skin lesion showed slight hyperkeratosis and parakeratosis and focal infiltration of lymphocytes, histiocytes and some eosinophils perivascularly and in the upper layers of the dermis.

(iii) Immunofluorescence (IF) studies: The standard procedures for DIF study and IF study and the microscopic system used have been described previously (Cormane & Szabó, 1970) as were those for the detection of complement and the characteristics of the anti-complement serum (van Joost, Cormane & Pondman, 1972). Highly specific FTC-labelled monovalent antisera against IgG, IgA, IgM, IgE and IgD were used in this study. The sera tested by IF test were absorbed previously with group A and B erythrocytes. Guinea-pig lip and normal human mucosa were used as substrates.

The sections of the ocular mucous membrane examined for DIF staining showed a bright band-like fluorescence of the subepithelial regions, but due to tissue degeneration and complete absence of the epithelium at these sites the interpretation of this staining was awkward. A conspicuous predominant linear deposition of only IgG and complement was seen along the basement membrane of the buccal mucous membrane (Fig. 2). DIF staining of the psoriasiform skin lesions and the normal light exposed skin was found to be negative.

The serum samples on IF examination did not contain basement membrane antibodies, but using guinea-pig lip as the substrate intercellular pemphigus-like antibodies (P-Lab) were demonstrable with a titre of 1:40. Two months after the practolol had been stopped these antibodies were no longer demonstrable.

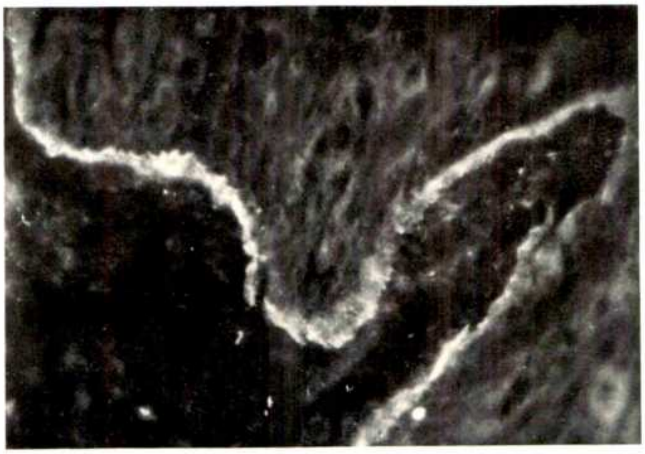

FIGURE 2. Oral mucous membrane. DIF technique (IgG/FIC × 500). Note the predominant linear fluorescent staining of the basement membrane zone.

DISCUSSION

The clinical and histopathological investigations on the conjunctivae were not typical for lupus erythematosus or for bullous pemphigoid, but accorded with the diagnosis of ocular cicatricial pemphigoid (Lever, 1965). The predominantly linear, but focally fine granular depositions of Ig and complement at the basement membrane zone *in vivo* (DIF) are usually demonstrable in bullous pemphigoid if an optimal fluorescence microscope is used (Cormane & Szabó, 1970). Recently several different authors using IF techniques (Bean *et al.*, 1972; Cram, Griffith & Fukuyama, 1974; Griffith, Fukuyama & Tuffanelli, 1974) have reported in cicatricial pemphigoid the same predominantly linear depositions of Ig and complement *in vivo* at the basement membrane. Only some authors (Dabelsteen, Ullman & Rygaard, 1974) have reported the concomitant occurrence of circulating pemphigoid antibodies in some but not in all cases of cicatricial pemphigoid.

In our patient some important features of drug-induced LE were demonstrable namely LE-cells and ANA in the absence of precipitating anti-DNA antibodies. In LE, however, a more coarse granular deposition of immunoglobulins and complement at the dermo–epidermal zone can be expected (Cormane & van Joost, 1973). Furthermore the positive DIF-results could only be obtained in the mucous membranes and not in the psoriasiform skin lesions or in the unaffected skin.

The significance of intercellular pemphigus-like antibodies (P-Lab) in our case and in the cases associated with practolol therapy recently described by Amos, Brigden & McKerron (1975) is still unknown. It is noteworthy that P-Lab have been reported in cicatricial pemphigoid (Cram *et al.*, 1974) as well as in drug reactions (Fellner *et al.*, 1973; van Joost, 1974).

It can be concluded that as well as other complications an ocular syndrome with clinical, histological, and immunological features similar to those described in cases of ocular cicatricial pemphigoid can result from long-term use of practolol. Further studies are required to cast more light on the role of drugs in the immunopathology of bullous skin reactions.

REFERENCES

AMOS, H.E., BRIGDEN, W.D. & MCKERRON, R.A. (1975) Untoward effects associated with practolol: demonstration of antibody binding to epithelial cells. *British Medical Journal*, **i**, 598.

BEAN, S.F., WAISMAN, M., MICHEL, B., THOMAS, CH. J., KNOX, M. & LEVINE, M. (1972) Cicatricial pemphigoid: immunofluorescent studies. *Archives of Dermatology*, **106**, 195.

CORMANE, R.H. & SZABÓ, E. (1970) Immunohistopathology of the skin in pemphigoid. *British Journal of Dermatology*, **83**, 45.

CORMANE, R.H. & VAN JOOST, Th. (1973) Lupus erythematosus: immunologic studies of the skin. In: *Immunopathology of the skin: labelled antibody studies.* (Ed. by E.H.Beutner, T.P.Chorzelski, S.F.Bean and R.E. Jordan) Chpt. 7. Dowden, Hutchinson & Ross, Stroudsburg, Pennsylvania.

CRAM, D.L., GRIFFITH, M.R. & FUKUYAMA, K. (1974) Pemphigus-like antibodies in cicatricial pemphigoid. *Archives of Dermatology*, **109**, 235.

DABELSTEEN, E., ULLMAN, S. & RYGAARD, J. (1974) Demonstration of basement membrane autoantibodies in patients with benign mucous membrane pemphigoid. *Acta dermato-venerologica (Stockholm)*, **54**, 189.

FELIX, R.H., IVE, F.A. & DAHL, M.G.C. (1974) Cutaneous and ocular reactions to practolol. *British Medical Journal*, **iv**, 323.

FELLNER, H.J., FUKUYAMA, K., MOSHILL, A. & KLAUS, H.V. (1973) Intercellular antibodies in blood and epidermis. *British Journal of Dermatology*, **89**, 115.

GRIFFITH, M.R., FUKUYAMA, K. & TUFFANELLI, D. (1974) Immunofluorescent studies in mucous membrane pemphigoid. *Archives of Dermatology*, **109**, 195.

HUNT, D. & FREW, J.L. (1975) Pericarditis and peritonitis associated with practolol therapy. *British Medical Journal*, **i**, 92.

LEVER, W.F. (1965) *Pemphigus and pemphigoid.* Charles C.Thomas, Springfield, Illinois.

RAFTERY, E.B. & DENMAN, A.M. (1973) Systemic lupus erythematosus induced by practolol. *British Medical Journal*, **ii**, 452.

ROWLAND, M.G.M. & STEVENSON, C.J. (1972) Exfoliative dermatitis and practolol. Letter to the Editor. *Lancet*, **i**, 1130.

VAN JOOST, TH., CORMANE, R.H. & PONDMAN, K.W. (1972) Direct immunofluorescent study of the skin on occurrence of complement in pemphigus. *British Journal of Dermatology*, **87**, 466.

VAN JOOST, TH. (1974) Incidence of circulating antibodies reactive with basal cells of skin in drug reactions. *Acta dermato-venerologica (Stockholm)*, **54**, 183.

WRIGHT, P. (1974) Skin reactions to practolol. *British Medical Journal*, **ii**, 560.

WRIGHT, P. (1975) Untoward effects associated with practolol administration: oculomucocutaneous syndrome. *British Medical Journal*, **i**, 595.

British Journal of Dermatology (1976) **94**, 451.

Contact dermatitis from propolis

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SUMMARY

Two patients with contact dermatitis due to the natural product propolis (bee glue) are reported. They presented perioral eczema and stomatitis which were recalcitrant until propolis was considered as the cause. Patch tests with propolis preparations were positive in both patients, and, furthermore, in the second patient the lesions relapsed after provocation tests. European standard patch tests including balsam of Peru were negative. The complexity of propolis, its supposed anti-inflammatory effect due to flavonoids, and the sensitizing agents originating mainly from the poplar trees are discussed together with the cross-sensitization to balsam of Peru.

Contact dermatitis due to propolis should be considered in unexplained eczemas, mainly perioral but also in other areas, as propolis preparations are available also as ointments and cosmetic creams.

In many Western European countries, notably Scandinavia, Germany, Great Britain and Holland, propolis or bee glue has been known for many years. As a natural product it is sold now without prescription for several purposes as tablets, solid propolis in large or small pieces, powder, ointment, and recently as cosmetic moisture creams.

Beekeeper's dermatitis due to propolis is well known (Umansky, 1934; Börlin, 1947; Rothenborg, 1967; Bunney, 1968), and Winkler (1956) noted the apparent association between sensitivity to propolis and to poplar resins. By contrast, contact dermatitis due to propolis in persons without contact with poplar trees and in non-beekeepers has not been described previously. With increasing use of propolis it will probably be encountered more frequently by dermatologists. Furthermore, recalcitrant often periorally located eczema may remain unexplained until propolis as a cause is considered.

At Rigshospital, we have seen recently two patients with contact dermatitis due to propolis.

CASE REPORTS

Case 1

A woman, aged 59 years, who had suffered from dry lips for many years. In 1968 she had been treated for contact dermatitis due to lipstick. During August–September 1974 she chewed, drank and ate propolis for mental depression and tiredness. After this period she continued to eat the honey. In

October she noticed that her lips became unusually dry, stiff, and crusted. She did not use lipstick during these months. In an attempt to improve the condition, she tried to apply propolis honey directly to her lips. However, this caused severe pain and a burning sensation. The discomfort continued, and her lips became red and swollen despite the fact that she was treated with a corticosteroid cream. At this time the patient revealed to the dermatologist, who had treated her for 3 weeks, that all the time she had been eating propolis honey daily. Patch testing with granular propolis was subsequently performed, and a positive reaction was observed 2 days later. She was admitted to our department for recalcitrant cheilitis.

On admission, an acute oozing and crusted eczema was found around her mouth and on her chin. On her back, a positive patch test from granular propolis, applied 7 days earlier, was still visible. Other patch tests including balsam of Peru were negative. The intake of propolis was stopped before admission. Her eczema cleared in a few days on treatment with carbowax 1500 containing 10% propylene glycol, lidocain ointment and a corticosteroid cream.

Case 2

A woman, aged 67 years, who had suffered from Menière's disease and migraine for many years. From 1969 to 1972 she was treated for ano-perineal prurigo and genital candidiasis. In 1972, a hysterectomy with salpingo-oophorectomy was performed for cancer. After this operation she suffered from vaginal fluor, which, according to her statement, disappeared completely within 1–2 months of using propolis.

In August 1974 she started taking one tablet of propolis daily and chewing propolis for a gingival infection. Because of increasing soreness of the gums, chewing had to be given up now and then. As the gingival infection was unimproved, she tried to apply chewed propolis directly in the alveolo-buccal sulci, as recommended in leaflets from the producers. However, this resulted in oedema, erythema and severe pain.

Three days before she was seen in the hospital she stopped taking propolis with slight improvement. On examination two aphthae and slight erythema and oedema of the mucous membrane were found in the right inferior alveolo-buccal sulcus. Patch tests with propolis honey, ointment 5% and granular propolis in water were positive after 48 and 72 h.

In April, i.e. 3 months after the first examination, the patient returned for a provocation test. In the meantime she had continued to take propolis tablets for 1 month. Before provocation, slight erythema was seen on the inferior gingiva corresponding to the two middle and the right lateral incisors (Fig. 1). Apart from this, the mucous membranes were normal. Granular propolis, chewed for 1 min and placed in the inferior alveolo-buccal sulcus for 1 h for provocation, was used. The following evening the procedure was repeated and the time extended to 2–3 h resulting in severe pain, difficulty with talking, and in blisters. A planned provocation test on the third evening could not be performed because of the pain. Oedema, erythema, and a 6 × 7 mm white blister were found on examination on the fourth day (Fig. 2). The patient herself had made a patch test on her arm, which was positive.

DISCUSSION

Such patients with perioral eczema and stomatitis would escape an aetiological diagnosis unless the clinician is aware of the fact that propolis can give rise to contact dermatitis. In both cases propolis was mentioned by chance, and both patients believed that a preparation sold without prescription ought to be harmless. The diagnosis was confirmed by a positive provocation test in one patient, and patch tests were positive in both patients. It is well known that propolis causes allergic and not toxic patch test responses (Umansky, 1934; Börlin, 1947; Rothenborg, 1967; Bunney, 1968).

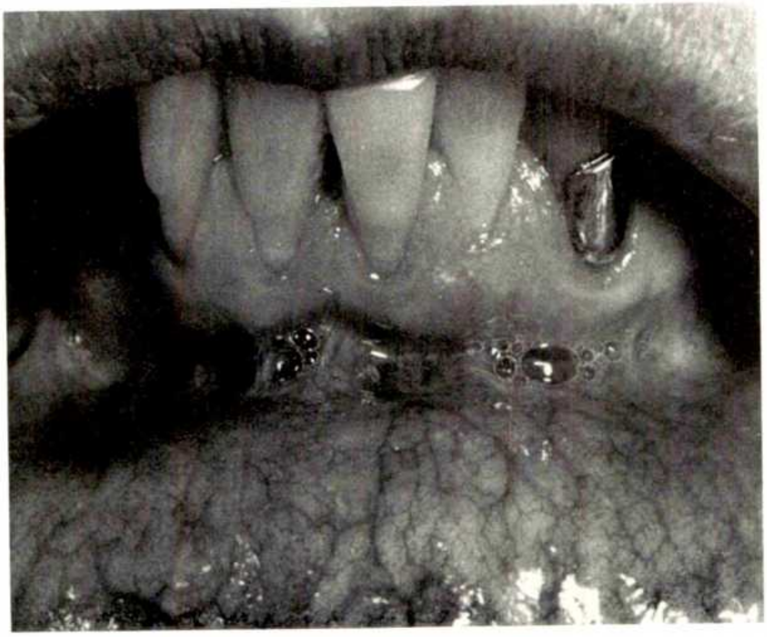

FIGURE 1. Patient 2 before provocation.

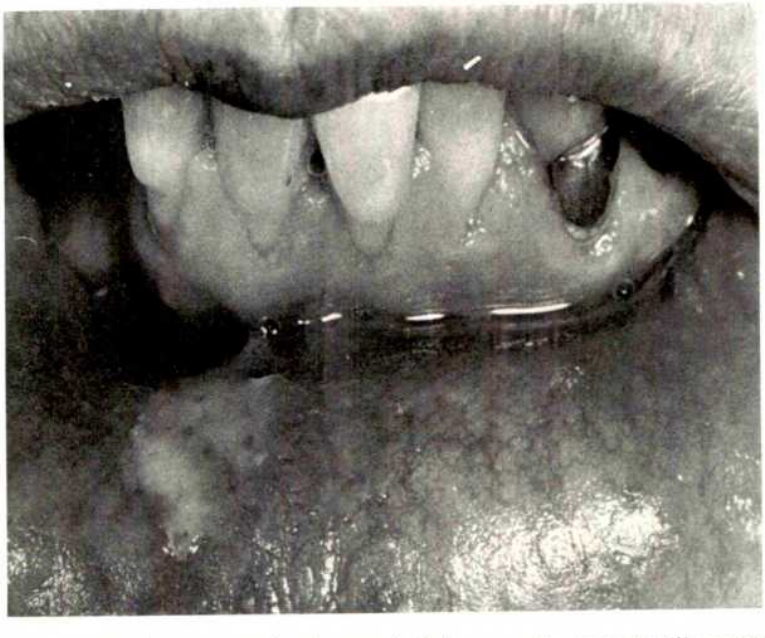

FIGURE 2. Patient 2 after provocation (see text). Oedema, erythema and a blister were seen.

In Great Britain, several cases of contact dermatitis in beekeepers in contact with propolis have been described, and the incidence of the dermatitis among beekeepers is estimated to be 0·05% (Bunney, 1968). With increasing use of propolis, more cases of contact dermatitis must be expected.

Propolis, which means 'before city', is a dark yellowish-brown resinous substance with strong adhesive properties. Mixed with wax, it is used by the bees to seal up crevices in the hives, to smooth the surfaces and to protect the entrance from intruders. It is collected by the honeybees from the resinous exudations of plants and trees such as horse-chestnut (*Arbor castanea*), spruce (*Abies nobilis*), willow (*Salix*), larch (*Larix*), fir (*Pinus*), and especially poplar trees (*Populus*). Owing to the different places of origin, propolis specimens vary. However, ordinarily it contains wax 30%, resins and balsams 55%, essential oils 10%, pollen 5%, and cinnamic alcohol, minerals and flavonoids (B.R.A.; Umansky, 1934; Villaneuva *et al.*, 1964, 1970).

Since ancient times, antiseptic and healing properties in veterinary and in human medicine have been attributed to propolis. Russian dentists have called propolis a better anaesthetic than cocaine, and in Russia it is used as toothpaste, skin cream and toilet lotions. An antibacterial and antifungal action is also attributed to propolis (Lindenfelser, 1967; Lavie, 1968) and it has been found to improve some skin diseases (Molnar-Toth, 1965).

The main effect—if any—of propolis is supposed to be due to the flavonoids. The mechanism is considered to be a stabilization of the connective tissue and the vessels through protection of vitamin C against oxidation, which may be of some importance in the formation of collagen and in the synthesis of steroid hormones. Stabilization is caused also by the flavonoids by a decreased activity of the mucopolysaccharide-destroying enzymes (Niebes & Laszt, 1971). The effect of this stabilization could prevent the spread of infection.

With the complex composition of propolis, a great number of sensitizing agents should be considered. Bunney (1968) carried out patch tests with propolis from different sources and with poplar and other tree resins. She found that ten of thirteen patients reacted to propolis and fifteen of fifteen patients reacted to poplar resin extracts. Also, Rothenborg (1967) found that his patients sensitive to poplar resins reacted to propolis, which suggested that the sensitizing allergen in propolis is derived mainly from the poplar tree resins. Similarly, Winkler (1956) found, in two patients both in contact with popular trees and not with bees, positive patch tests to poplar resin as well as to propolis, suggesting that the allergen is the same.

Several authors (Börlin, 1947; Rothenborg, 1967; Bunney, 1968) have shown cross-sensitivity to balsam of Peru, assuming that there must be some common sensitizing agent in propolis and balsam of Peru. However, neither of our patients had a positive patch test to balsam of Peru. Hjorth (1961) reported that poplar buds contain esters of cinnamic alcohol, and he found a positive reaction to an extract of poplar buds in three of fourteen patients with a positive patch test to balsam of Peru. As propolis contains cinnamic alcohol (Umansky, 1934) and balsam of Peru contains cinnamic acid, a cross-reaction between the various cinnamic derivates is a possibility. However, in her fifteen patients, Bunney (1968) found none positive to balsam of Peru and only one positive to the cinnamic derivates tested. Among five persons positive to poplar extracts, Rothenborg found one positive to cinnamic derivates and two to balsam of Peru.

Thus, it is generally believed that the allergens in propolis are mainly derived from the poplar trees, whereas the significance of the cinnamic derivates is not yet solved.

The conclusion is that in patients with recalcitrant, unexplained, periorally located eczema, contact dermatitis due to propolis should be considered as a possibility. It should be considered also in eczemas occurring in other areas because of the increasing use of propolis preparations as ointment and cosmetic moisture creams, the latter having been introduced recently onto the market.

REFERENCES

B.R.A. (Bee Research Association) (1973) *Research News No.* 9. Reprinted from *Bee World*, **54**, 71.

BUNNEY, M.H. (1968) Contact dermatitis in beekeepers due to propolis (Bee Glue). *British Journal of Dermatology*, **80**, 17.

BÖRLIN, E. (1947) Berufsekzeme bei Bienenzuchtern. *Dermatologica*, **94**, 109.

HJORTH, N. (1961) Eczematous allergy to balsams, allied perfumes and flavouring agents. *Acta dermato-venereologica (Stockholm)*, **41**, Suppl. 46.

LAVIE, P. (1968) *Traité de biologie de l'abeille* (Ed. by R.Chauvin), Vol. 3, p. 1. Masson et Cie, Paris.

LINDENFELSER, L.A. (1967) Antimicrobial activity of propolis. *American Bee Journal*, **107**, 90, 130.

MOLNAR-TOTH, M. (1965) Therapeutische Ergebnisse bei Anwendung von Propolis bei verschiedenen Hautkrankheiten. *Apimondia* der 20. *Internationale Jubiläumskongress der Bienenzuchter*, III, **37**, 1.

NIEBES, P. & LASZT, L. (1971) Influence *in vitro* d'une série de flavonoïdes sur des enzymes du métabolisme des mucopolysaccaharides de veines saphènes humaines et bovines. *Angiologica*, **8**, 297.

ROTHENBORG, H.W. (1967) Occupational dermatitis in beekeeper due to poplar resins in beeswax. *Archives of Dermatology*, **95**, 381.

UMANSKY, G.I. (1934) Über eine professionelle Dermatitis, verruascht durch Bienenharz Propolis. *Dermatologische Wochenschrift*, **98**, 177.

VILLANEUVA, V.R., BOGDANOVSKY, D., BARBIER, M., GONNET, M. & LAVIE, P. (1964) Sur l'isolement et l'identification de la 3, 5, 7 trihydroxy flavone (Galangine) a partir de la Propolis. *Annales de l'Institute Pasteur*, **106**, 292.

VILLANEUVA, V.R., BARBIER, M., GONNET, M. & LAVIE, P. (1970) Les flavonoïdes de la Propolis isolement d'une nouvelle substance bactériostatique: La pinocembrine [dihydroxy 5, 7 flavonone]. *Annales de l'Institut Pasteur*, **118**, 84.

WINKLER, A. (1956) Dermatitis durch Pappelharz. *Dermatologische Wochenschrift*, **133**, 81.

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History of Dermatology

Secondary skin cancer in nineteenth-century Britain

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SUMMARY

Clinico-pathological data on secondary skin cancer are of current interest. This paper therefore reviews such data with special reference to nineteenth-century Britain. The old authors were interested in such diverse aspects of the problem of skin secondaries as the mode of invasion, the forms of presentation, the patterns of distribution, clinical diagnosis, microscopic diagnosis, prognosis, and treatment. Although skin secondaries were of serious prognostic import, all cases showing them were not hopeless.

Recently, Brownstein & Helwig (1973) reviewed the clinical and pathological features of secondary tumours of the skin. In the present paper, I wish to consider these features anew but with special reference to nineteenth-century Britain.

During the nineteenth century, first France and then Germany produced the first real pathologists, i.e. men who devoted themselves full-time to pathology. Thus, as Long's (1928) *History of Pathology* shows, early pathologists famed in the field of tumours were mostly continentals like Cruveilhier (1791–1873), Virchow (1821–1902), Billroth (1829–94) and Waldeyer (1836–1921). In Britain, on the other hand, there were originally the good physicians and surgeons who were interested in pathology and made some contributions to it. Among them may be mentioned Thomas Hodgkin (1798–1866) whose cancer carriage theories remain interesting (Onuigbo, 1967). However, towards the end of the century, British pathologists became prominent on the oncological scene. One of them was Joseph Coats (1846–99), whose own theories are also of current interest (Onuigbo, 1958). This review will be wholly devoted to British dermatology *vis-à-vis* cancer metastasis.

Some general remarks are necessary. According to Singer & Underwood (1962), it was in about 1650 that a way was found for mounting simple lenses in order to magnify tiny objects. This led to the seventeenth-century discoveries made by such stalwarts as Marcello Malpighi (1628–94) and Antoni van Leeuwenhoek (1632–1723). However, effective instrumentation occurred only during the nineteenth century. Indeed, the great leap forward in microscopy took place in the 1830s. As discussed elsewhere (Onuigbo, 1963), following the triumphant ascendancy of the microscope as a research tool, the old authorities took some time to accept the cellular theory of cancer metastasis.

'With the evolution of the microscope in the nineteenth century', wrote Russell (1908) in his Aberdeen doctorate thesis, 'at first botanists and later zoologists were able to observe carefully the histological structure of living tissues, and this served as a basis on which the embryologists and the pathologists were respectively able to follow out in detail the normal and abnormal processes of growth'. In the words of Cappell (1931): 'the study of cancer lay in the fog of the humoral theories until the classical researches of Schwann and of Müller [in 1838] introduced the conception of cellular structure, which culminated in the great work of Virchow with his dictum *Omnis cellula e cellula.*' However, even Virchow himself was tardy in accepting the cellular nature of metastasis (Onuigbo, 1962).

Advances in microscopy occurred throughout the nineteenth century (Singer & Underwood, 1962). In 1841, John Hughes Bennett (1812–75) started to lecture on histology at Edinburgh University and included microscopical demonstrations of morbid histology. By the 1850s, the microtome had been invented and tissue sections of desired thinness could be obtained. From about 1870, the introduction of staining methods improved the appearances. Thereafter, pathological anatomy was inseparably linked with morbid histology. The latter's importance as a diagnostic aid was appreciated by Hutchinson (1881) and other surgeons.

In 1853, Paget asked, 'Has the microscope discovered any structure which is decisive of cancer, wherever it is found?' Throughout the rest of the century, different attempts were made so to improve techniques as to be able to answer this and related questions. For example, when Thin (1876) presented his researches on histological changes in cancer of the skin before the Royal Medical and Chirurgical Society, he mentioned the mode of preparation, 'from sections of the tumours which had been put fresh in solution of osmic acid'. In his view, this solution had 'advantages over any other method at present known'.

In sum, a review of secondary tumours of the skin as they appeared in British writings of the nineteenth century should be read with due attention to the changing circumstances of those days. In fact, as late as 1893, Power considered that the first step in the rational study of cancer should consist of research on the microscopical changes which simple irritation can cause on the skin!

LITERATURE

The occurrence of secondary skin cancer was known early in the nineteenth century. But, as Home (1805) pointed out, the mode of invasion itself was not understood in those days.

Two decades later, Travers (1829) stated that the local skin becomes 'charged with the (cancerous) poison' because the lymphatics are 'numerous in this texture'. Budd (1845) mentioned Sir Astley Cooper's beautiful demonstration of the fact that the satellite skin nodules of breast cancers of long standing are seated in the lymphatics and added that the nodules may be found in retrograde positions after lymphatic blockage. In his Aberdeen doctorate thesis, Galloway (1892) wrote that remote skin deposits follow the course of the collateral lymph circulation. Battle (1895) demonstrated—with a surgical specimen of melanoma of the clitoris—the picturesque permeation of the lymphatics of the pubic skin.

Finlay (1883a), who reported a case of gastric carcinoma with multiple metastases in the skin and other parts, argued that 'it seems most reasonable to look to the blood as the agent in the dissemination of the cancerous infection to the remoter structures'. Beadles (1894) accepted this mode of spread to distant skin.

A form of secondary skin cancer is that due to infiltration from subjacent tumours (Walshe, 1871; Williams, 1889). In Bell's (1816) patient, for example, testicular tumour infiltrated the overlying scrotal skin. However, the offending primary may be quite deeply seated as in the lung cancer case presented by Finlay (1885). The skin may, moreover, be involved from a secondarily invaded tissue, e.g. groin skin from replaced groin nodes in cases of scrotal cancer (Hutchinson, 1881).

A peculiar form of spread is the extensive surface type. In the 'superficial and herpetic' form of breast cancer, according to Travers (1829), the growth 'creeps upon the surface, and converts the entire front or side of the chest into a warty or tubercular ulcer'. 'In such cases', he continued, 'it is very usual for the opposite breast to become similarly affected.' In Coupland's (1876) case, the skin covering the upper part of the chest 'was thickly beset by firm adherent nodules', while that covering the opposite breast 'was much thickened and milky-white in appearance'.

The form of skin involvement which most interested the old masters was perhaps that seen in mastectomy cicatrices (Coote, 1846; Andrew, 1870; Bennett, 1871; Williams, 1889). According to Home (1805), cicatricial deposits were commoner after palliative surgery. Such a deposit may occur soon after or even before the cicatrix forms (Travers, 1829). In the opinion of Paget (1853), it was met with 'very commonly'.

Iatrogenic skin secondaries were recognized by Fox (1891) and others. Lack (1896) spoke of 'the great danger we run in infecting our patient with the very disease we are endeavouring to eradicate if at any time during the operation we cut into the affected tissues or infect our hands, etc., with it'. He exemplified, among others, with a woman whose ovarian tumour burst during removal. He wrote that 7 months later: 'The peritoneum over the old abdominal scar was healthy, but each of the deep stitch tracks was the site of a tumour varying in size from a pea to a cherry stone. These little tumours formed a double row, and on microscopic examination proved to be cylindrical-celled carcinomata'. Macewen's (1886) report was also apt: 'In another case of carcinoma of the bladder, in which perineal section had to be resorted to to relieve the pain consequent upon distension of the walls of the viscus, an abrasion accidentally occurred over the course of the T bandage by which the canula was kept *in situ* and several nodules of carcinoma grew in the course of the abrasion, the cells having been carried thither by the constant welling away of the urine.'

Contact cancer interested the old observers, Macewen (1886) citing several examples. Its literature was reviewed by Lack (1896). His illustrations included two cases in which vulval cancer 'was followed by a similar growth on the parts in contact on the opposite side'.

Skin secondaries were noted to have patterns. The nodules were localized (Macewen, 1886; Steven, 1891) or generalized (Dickinson, 1863; Kidd, 1883; Coats, 1885). In the latter cases, the tendency for the growths to preponderate on, or to be limited to, the chest and abdomen was noticed by Sanderson (1855), Ogle (1856), Andrew (1870) and Mackenzie (1891). In the patient described by Ogle (1858), the deposits occurred mainly on the left side of the trunk, whereas in Finlay's (1883a) patient they were more numerous on the front of the trunk than on the back. In some patients with numerous nodules, it was remarkable that certain parts were spared, e.g. the leg (Finlay, 1883a) and the face (Mackenzie, 1891). Relationship to the umbilicus was appreciated by Dickinson (1863), Coupland (1877) and Finlay (1883a). Of the nodules seen by Beadles (1894), one was specifically stated to be related to a distant old scar. Uncommon recorded sites of invasion included the forehead (Stokes, 1837) and perineum (Rolleston, 1899).

Differential diagnosis received the attention of the old authorities. Thus, Bell (1816) differentiated, on grounds of appearance, between mere inflammatory ulceration of the skin and actual malignant ulceration from underlying growths. Walshe (1846) also contrasted both forms of skin ulcers.

Lung cancer provided problems of differential diagnosis. Graves (1834) confessed that he was misled by a case presenting with skin secondaries for he mistook them for tuberculous deposits. Shortly after, Stokes (1837) emphasized the importance of such secondaries when he offered 21 clues to the diagnosis of this cancer. However, two decades later, Bell (1857) could not but suspect that such external chest deposits were 'seldom or never taken into consideration by physicians or surgeons'. But, by 1871, Walshe was able to assert that this tumour migrates relatively frequently to the skin over the chest.

The multiform picture of metastatic melanoma was recognized. Ogle (1858) described a man from whom a skin tumour had been removed over 7 years previously from the right hypochondriac region; subsequently, melanotic nodes were excised from the right groin, and then skin nodules appeared in many other parts. Over a hundred such nodules were counted by Arnott (1869) in a woman whose small black wart was exised from the forearm about a year previously. In contrast, only a few skin deposits were found by Godlee (1874) when he necropsied a man who died on the 14th post-operative day as an aftermath of drinking 'two medicine-bottles full of wine which he had smuggled into the hospital'.

Although patients may present with skin nodules (Dickinson, 1863; Finlay, 1883a), it is noteworthy that in many of the recorded cases these tell-tale nodules cropped up or evolved further following hospitalization (Arnott, 1869; Andrew, 1870; Coupland, 1877; Macewen, 1886; Beadles, 1894). For example, among the more important happenings during their patient's stay in the hospital, according to Calvert & Pigg (1898), was the appearance of some subcutaneous secondaries on the chest.

The condition of the patients themselves varied. Whereas some came to hospital in an emaciated state (Finlay, 1883a) or very ill state (Targett, 1891), it was remarkable that nodules were often found in tolerably healthy patients (Dickinson, 1863; Arnott, 1869; Godlee, 1874; Coupland, 1876). Incidentally, in Arnott's (1869) case, the patient purposely failed to reveal their presence for fear of 'further cutting'.

Clinical examination of skin nodules provided a wealth of information. The skin over the growths varied from natural colour (Stokes, 1837) to purplish colour (Ogle, 1858; Dickinson, 1863). The skin was either adherent to, or movable on, the nodule (Sanderson, 1855; Dickinson, 1863; Finlay, 1883a). In this connection, Stokes (1837) stated that it was movable at first and more fixed afterwards. Nodules evolved rapidly (Stokes, 1837; Coupland, 1876) and varied much in size (Arnott, 1869; Finlay, 1883b; Mackenzie, 1891). The overlying skin may ulcerate (Dickinson, 1863; Arnott, 1969) or not (Finlay, 1883a). Likewise, pressure on the nodules may elicit tenderness (Dickinson, 1863) or not (Bennett, 1871). Moreover, the underlying bone may be eroded (Arnott, 1869) or not (Mackenzie, 1891).

Finlay (1883a) biopsied two subcutaneous nodules from a man in whom clinical examination disclosed no primary neoplasm. Microscopy revealed acini with columnar epithelium. On this account, he wrote aptly: 'It may be mentioned as a fact of clinical interest, that from the structure of the nodules excised during life, it was concluded that the primary growth was in the alimentary tract, although its exact locality could not be fixed.' At necropsy, a gastric growth materialized.

Before the dawn of microscopy, naked eye appearances of skin secondaries were naturally well known. After microscopy came into common use, macroscopic descriptions flourished still. A good example comes from Finlay (1883a) who found skin nodules to be 'hard and white, with red striae radiating from their centres'. 'Their cut surfaces', he added, 'yielded a little milky juice on scraping.'

Microscopical confirmation of skin secondaries after necropsy was recorded by Coupland (1877), Macewen (1886), Mackenzie (1891), and Rolleston (1899). Sanderson (1855) even compared the skin and other deposits which he had obtained at necropsy. 'The cells which occurred in the tumours of the skin', he remarked, 'differed from the others, in being fusiform instead of oval.' Dickinson (1863) combined his description: 'On section, they had all the characters of encephaloid; and under the microscope, the cells were of the rounded variety found in tumours of that nature.' Godlee (1874) encountered these round cells as well as spindle cells. Finlay (1883b) confessed that he was unable to examine the collected nodule due to its being mislaid. Beadles (1894), whose sections were stained with logwood after being cut with the ether-freezing microscope, gave a detailed account of several skin nodules examined: 'The smaller growths are situated entirely in the subcutaneous connective tissue. They are of round form, and the growth is distinctly limited except at the superficial side, where there is a slight tendency to invade the cutis vera. There is a fairly definite alveolar arrangement formed by connective-tissue bands passing in from the periphera. In places this stroma is more or less hyaline in character, and in other parts where this is less marked it contains numerous small blood-vessels. The alveolar spaces are occupied by cells which stain and have the character of cells derived from epithelium. They are for the most part of more or less uniform size, larger than the natural cells of the rete mucosum.... In some cases the cell masses have spread upwards and are lying in the cutis vera more immediately under the epidermis, but in none of these smaller nodules where ulceration does not exist can any connection be traced between the growth and the cells of the rete. The hair-follicles and the sweat-glands also appear healthy and do not communicate with the new growth. In some of the sections veins of considerable size, situated in the lower part of the cutis and in the subcutaneous tissue in the neighbourhood of the nodule, are filled with malignant cells.'

Prognosis after the appearance of skin nodules was generally poor. Death usually occurred within 3 months (Andrew, 1870; Coupland, 1876; Finlay, 1883a; Rolleston, 1899). However, some patients lived up to 4 months (Dickinson, 1863; Calvert & Pigg, 1898) or even 6 months (Arnott, 1869; Beadles, 1894).

What was probably a form of active treatment was reported by Beadles (1894). He wrote as follows: 'On March 6th, 1891, the patient was admitted into the Cancer Hospital with numerous growths in the skin. The smaller ones had appeared since Christmas. He had been losing flesh. Between March 6th and 12th several subcutaneous injections of methyl violet were given into the nodules on the scalp.' In his review, Lack (1896) included a case each of laryngectomy and pylorectomy in which secondary deposit in the operation scar was subjected to excisional treatment.

Hutchinson (1881) taught that the excision of skin deposits associated with mastectomy cicatrix should be 'promptly undertaken'. Coupland (1876) carried this out in a case seen 5 months after the initial mastectomy, while Williams (1889) recorded not only instances of surgical excision but also those of destruction with either caustics or cautery. One of his cases concerned a man whose breast was amputated for carcinoma. Four months later, growth was found in the opposite breast. This was then amputated. There was no local recurrence but, 27 months after the second operation, a nodule formed in the skin of the hand and 'was successfully excised'. Another of his case reports merits quotation:

'Soon after amputation of the left breast for primary cancer, recurrence was noticed in the chest. In the course of 10 years it increased to the size of an orange, and several discrete hard nodules developed at the axillary end of the scar, but there was no obvious affection of the axillary glands. At the end of this time the recurrent disease was excised. There was no subsequent disease in the chest and axilla; but 11 years afterwards the patient died with metastatic growths in the liver and in the cardiac part of the stomach.'

In conclusion, the above gastric growth was perhaps another neoplasm altogether, its own hepatic deposits occurring in a long-lived patient cured of both the original breast primary and its contiguous skin secondaries. Conjecture apart, this old case confirms the hopeful note with which Brownstein & Helwig (1973) ended their recent review of secondary skin cancers: such cancers are indeed of serious prognostic import, but all cases are not hopeless, long survival being possible.

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REFERENCES

Andrew, J. (1870) Disseminated cancer. *Transactions of the Pathological Society of London*, **21**, 2.

Arnott, H. (1869) Multiple melanotic tumours. *Transactions of the Pathological Society of London*, **20**, 322.

Battle, W.H. (1895) Primary melanotic sarcoma of clitoris. *Transactions of the Pathological Society of London*, **46**, 189.

Beadles, C.F. (1894) A case of multiple malignant growths. *Transactions of the Pathological Society of London*, **45**, 188.

Bell, C. (1816) *Surgical Observations*, pp. 399–402. Longman, London.

Bell, J. (1857) Remarkable cases of malignant (encephaloid) disease. *Glasgow Medical Journal*, **4**, 415.

Bennett, J.R. (1871) Secondary scirrhous, or fibro-cancerous, infiltration of connective tissue of lung, etc.; primary cancer of mamma removed. *Transactions of the Pathological Society of London*, **22**, 76.

Brownstein, M.H. & Helwig, E.B. (1973) Spread of tumours to the skin. *Archives of Dermatology*, **107**, 80.

Budd, G. (1845) *On Diseases of the Liver*, p. 311. Churchill, London.

Calvert, J. & Pigg, T.S. (1898) A case of melanotic sarcoma. *Transactions of the Pathological Society of London*, **49**, 297.

Cappell, D.F. (1931) Observations on cancer of the breast in the light of experimental cancer research. *Glasgow Medical Journal*, **115**, 181.

Coats, J. (1885) *Catalogue of the Pathological Museum of the Western Infirmary, Glasgow*, pp. 77–81. Alex MacDougal, Glasgow.

Coote, H. (1846) Instances of melanosis, with observations. *Lancet*, **ii**, 142.

Coupland, S. (1876) Cancer of both breasts and ovaries. *Transactions of the Pathological Society of London*, **27**, 259.

Coupland, S. (1877) Lymphadenoma of the stomach, retro-peritoneal and mesenteric glands, kidneys, ovaries thyroid, and of the intermuscular tissue of the thigh and skin. *Transactions of the Pathological Society of London*, **28**, 126.

Dickinson (1863) Subcutaneous cancer, secondary to cancer of the glans penis. *Transactions of the Pathological Society of London*, **14**, 240.

Finlay, D.W. (1883a) Epithelioma of stomach with wide diffusion of secondary deposits in other viscera and throughout the integuments. *Transactions of the Pathological Society of London*, **34**, 102.

Finlay, D.W. (1883b) Fibro-myoma of the uterus, becoming sarcomatous, with secondary growths in pleura, heart and kidney. *Transactions of the Pathological Society of London*, **34**, 177.

Finlay, D.W. (1885) Case of lymphosarcoma of left lung with great displacement of heart. *Medical Times and Gazette*, **i**, 146.

Fox, W. (1891) *A Treatise on Diseases of the Lungs and Pleura*, p. 1164. Churchill, London.

Galloway, J. (1892) *The Malignant Diseases of Serous Membranes*, pp. 21–22. M.D. Thesis, Aberdeen University.

Godlee, R.J. (1874) Melanotic sarcoma in the medulla oblongata secondary to a similar growth situated probably in a lymphatic gland. *Transactions of the Pathological Society of London*, **25**, 18.

Graves, R.J. (1834) Observations on the treatment of various diseases. *The Dublin Journal of Medical Science*, **4**, 309.

Home, E. (1805) *Observations on Cancer with Connected Histories of the Diseases*, pp. 146 and 175. Longman, London.

Hutchinson, J. (1881) On the local origin of cancer. *Medical Times and Gazette*, **i**, 92.

Kidd, P. (1883) A case of disseminated cancer. *Transactions of the Pathological Society of London*, **34**, 240.

Lack, H.L. (1896) A contribution to the operative treatment of malignant disease of the larynx, with special reference to the danger of cancerous wound infection. *Lancet*, **i**, 1638.

Long, E.R. (1928) *A History of Pathology*, pp. 142–205. Williams & Wilkins, Baltimore.

Macewen, W. (1886) Carcinoma in its pathological and aetiological aspects, with remarks on the principles of its surgical treatment. *Glasgow Medical Journal*, **25**, 271.

MacKenzie, J.C. (1891) Melanotic sarcoma, very widely disseminated. *Transactions of the Pathological Society of London*, **42**, 321.

Ogle, J.W. (1856) Melanotic carcinomatous deposit connected with the dura mater, the lining of the ventricles, and the seventh and ninth pairs of cranial nerves. *Transactions of the Pathological Society of London*, **7**, 5.

Ogle, J.W. (1858) Carcinoma (chiefly the melanotic form) of the brain, as also of other parts. *Transactions of the Pathological Society of London*, **9**, 20.

Onuigbo, W.I.B. (1958) Joseph Coats (1846–99) of Glasgow and the theory of cancer metastasis. *Scottish Medical Journal*, **15**, 281.

ONUIGBO, W.I.B. (1962) The paradox of Virchow's views on cancer metastasis. *Bulletin of the History of Medicine*, **36**, 444.

ONUIGBO, W.I.B. (1963) A history of the cell theory of cancer metastasis. *Gesnerus*, **20**, 90.

ONUIGBO, W.I.B. (1967) Thomas Hodgkin (1798–1866) on cancer cell carriage. *Medical History*, **11**, 406.

PAGET, J. (1853) *Lectures on Surgical Pathology*, vol. 2, pp. 553 and 565. Longmans, London.

POWER, D. (1893) Some effects of chronic irritation upon living tissues, being first steps in a rational study of cancer. *British Medical Journal*, **ii**, 830.

ROLLESTON, H.D. (1899) Primary columnar-celled carcinoma of the tail of the pancreas. *Transactions of the Pathological Society of London*, **50**, 171.

RUSSELL, B.R.G. (1908) *Studies on Experimental Cancer*, p. 1. M.D. Thesis, Aberdeen University.

SANDERSON (1855) Melanotic cancer in various organs. *Transactions of the Pathological Society of London*, **6**, 324.

SINGER, C. & UNDERWOOD, E.A. (1962) *A Short History of Medicine*, 2nd ed., pp. 124–135 and 627–640. Oxford University Press, London.

STEVEN, J.L. (1891) The pathology of mediastinal tumours, with special reference to clinical diagnosis. *Glasgow Medical Journal*, **36**, 110.

STOKES, W. (1837) *A Treatise on the Diagnosis and Treatment of Diseases of the Chest*, part 1, pp. 370–384. Hodges & Smith, Dublin.

TARGETT, J.H. (1891) Secondary melanotic sarcoma of the bladder. *Transactions of the Pathological Society of London*, **42**, 214.

THIN, G. (1876) On some of the histological changes found in cancer of the skin or epithelioma, with special reference to the source of the newly formed epithelial cells. *British Medical Journal*, **i**, 412.

TRAVERS, B. (1829) Observations on the local diseases termed malignant. *Medico-Chirurgioal Transactions*, **15**, 195.

WALSHE, W.H. (1846) *The Nature and Treatment of Cancer*, pp. 14–15. Taylor & Walton, London.

WALSHE, W.H. (1871) *A Practical Treatise on the Diseases of the Lungs*, 4th edn, p. 516. Smith, Elder & Co., London.

WILLIAMS, W.R. (1889) Cancer of the male breast, based on the records of one hundred cases; with remarks. *Lancet*, **ii**, 310.

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Book Reviews

System of Ophthalmology, Vol. XIII. The Ocular Adnexa. Part I. Diseases of the Eyelids. Part II. Lacrimal, Orbital and Para-orbital Diseases. SIR STEWART DUKE-ELDER and P.A. MACFAUL (1975) London: Kimpton. Pp. 1–592, 593–1236. 1300 illustrations, 6 colour plates. Price £25.00. (The two volumes are not sold separately.)

The first volume of Sir Stewart Duke-Elder's System of Ophthalmology was published in 1958, and the 15th and last volume is in preparation. The outstanding merit of this remarkable work is well known to ophthalmologists, for the author succeeds in presenting a detailed and critical survey of the world literature in a clear and pleasantly readable style. Many dermatologists too are familiar with these massive volumes and have had occasion to refer at times to the two volumes on the diseases of the outer eye published in 1965. However it is in the present Volume (which consists in fact of two volumes) that Sir Stewart, assisted by Mr Peter MacFaul, covers the diseases of the eyelids, the eyebrows and the orbits, many of which are common ground to the opthalmologist and the dermatologist.

In the last few years several useful works have been published on 'dermato-ophthalmology' including the book of this title by Casanovas and Vilanova of Barcelona in 1967 and *Haut und Auge* by Korting of Mainz in 1969. The latter has been translated into several languages, including English. The present work differs from these earlier contributions in being still more comprehensive; the international literature is covered in immense detail and the lists of references which follow the account of each disease are exhaustive.

This is essentially a reference book for the medical school library but it is one to which the dermatologist will quite often turn for advice on difficult diagnostic or therapeutic problems concerning the eyelids. He will seldom search in vain for the authors have been commendably thorough. However in the interests of brevity the account of the non-ophthalmological aspects of some conditions has been compressed to such a degree that inaccuracies sometimes arise by implication.

Figure 382 shows a patient who undoubtedly has the Hallermann–Streiff syndrome, yet the legend beneath it describes it as 'Congenital Ectodermal Dysplasia'. The quality of this monumental work is such that the reviewer is inclined to suspect that this is a 'deliberate mistake' on the part of the learned authors, to give him something to criticise.

ARTHUR ROOK

Insect Allergy. CLAUDE A.FRAZIER (1969) St Louis: Warren H.Green Inc. Pp. xiv+493, 165 illustrations. Price £12.00.

It is not the usual practice of this Journal to review a book 6 years after its date of publication; however, this volume, which only recently reached the Editor, is sufficiently unusual to deserve a belated notice. The late Albert Touraine wrote a scholarly monograph entitled *Les Parasitoses animales de la Peau*, but until now no subsequent author has attempted to bring together the literature on the many arthropod that are of concern to the allergist and the dermatologist.

Dr Claude A.Frazier of North Carolina, U.S.A., is an allergist whose many papers on reactions to insects have made his name well known to dermatologists. In his preface he describes his book as 'a collection of available and up-to-date information'. After a short introductory chapter on arthropods and man, the main families of arthropods producing toxic or allergic reactions are discussed systematically; a well illustrated account of the morphology and life history is followed by a description of the mechanisms of biting, the clinical syndromes, treatment, and prevention and control. The book therefore summarizes a great deal of useful information, much of it not readily accessible.

The book's principal defect is its parochial approach to the literature of medical entomology; most of the numerous references are to American publications, and the titles of some of the very few cited papers in languages other than English are fancifully mutilated. The emphasis throughout the book is therefore inevitably focused on American problems, and the author has missed the opportunity of providing the authoritative international text which would be a welcome and an invaluable reference book. The book has a second defect, but only from the point of view of the dermatologist; over 120 pages are devoted to Hymenoptera (Bees, Wasps, etc.) and only 37 to Mites, leaving no space for such clinically important genera as Cheyletiella.

This, then, is essentially a book for the allergist practising in North America, and Dr Frazier must be congratulated on an effective compilation which must have involved a great deal of work. For the dermatologist, particularly if he practices in Europe, the book is somewhat disappointing, but it will, nevertheless, provide him with much interesting reading and a wealth of unfamiliar facts.

ARTHUR ROOK

Clinics in Rheumatic Diseases. Vol. 1, No. 1. Rheumatological Manifestations of Systemic Disease. Guest Editor E.G.L.BYWATERS (1975) London: W.B.Saunders Co. Ltd. Pp. 213, Illustrated. Published three times a year, subscription price £15.00 per annum.

This first issue of a new series has chapters on some unusual arthropathies and other rheumatological manifestations associated with systemic disease. Throughout the book, emphasis is placed on the rheumatological aspects of systemic diseases, where the authors consider the rheumatological aspects have been comparatively neglected in the past or where new data have accumulated. There are, therefore, chapters on the rheumatological manifestations of, for example, heritable disorders of connective tissue, sickle cell disease and other haemoglobinopathies and haemophilia. The editor contributes an excellent chapter on Jaccoud's Syndrome and there are other chapters on amyloid disease, the lipidoses and familial Mediterranean fever.

From this it will be seem that it is a useful book for review articles of a number of rare conditions, in particular with regard to their rheumatological aspects. Therefore, each chapter cannot be considered as definitive, but there are good lists of references for most of the conditions, as a guide to further reading. There are some good tables, and a number of illustrations of variable quality.

For dermatologists the descriptions of the dermatological aspects are no better than may be found in a number of standard textbooks. However, for those with special interest in the rheumatic diseases this book provides interesting reading and up-to-date information to a degree that is helpful for the reader who is not a rheumatologist.

R.S.WELLS

Die Haut und Ihre Erkrankungen im Kindesalter. KLAUS DIETEL (1974) Leipzig: George Thieme. Pp. 258, 79 illustrations, 25 colour plates.

Dr Dietel, who is Chefarzt of the children's clinic at the District Hospital, Karl-Marx-Stadt, Upper Saxony, is to be congratulated on a remarkably comprehensive synopsis of skin disease in childhood which he has accomplished in less than 220 pages of text. He has also provided no fewer than 26 pages of references to the recent literature in German and English, both paediatric and dermatological. It should not escape the notice of dermatologists that the author is primarily a paediatrician; this not only reflects the increasing importance of a knowledge of dermatology for the paediatrician, but the relative neglect of paediatric dermatology by dermatologists as a group. A very helpful feature of the book for the practising clinician is the provision of 36 tables displaying the principal differential diagnostic features of the commoner and important childhood skin diseases. Dr Dietel deals succinctly with the definition, aetiology, pathogenesis and clinical features of each disease and discusses the treatment and management of each. There are, of course, details of pathogenesis and taxonomy which some dermatologists would dispute and his treatment on the whole reflects, as so often is the case, the current fashion so that it is not surprising to find simpler and more old-fashioned remedies rather than the more sophisticated and sometimes more hazardous treatment methods with which we are familiar in Western Europe and America.

However, this in no way detracts from the value of Dr Dietel's contribution. The black and white illustrations although small in size are representative of the conditions they display, but the colour reproductions are not of the same quality.

E.J.MOYNAHAN

Roxburgh's Common Skin Diseases. PETER BORRIE (1975) London: H.K.Lewis. Pp. 528, 239 illustrations. Price £6.00.

What can one say about the 14th Roxburgh? Perhaps that it is more like the 14th Earl of Home than the 14th Mr Wilson. Even more conservative than Sir Alec, it has not yet shed its title though Dr Borrie has been in charge for the last 4 editions and 16 years.

I found it rather a pleasant change to read a book in which the latest flash-in-the-pan theories have not been given a false significance simply because they are new. There are few surprises, once one gets past the elaborate 'touch discs' drawn in the epidermis on the first page and, although continuous rewriting may have dimmed the sparkle a little, the presentation is always clear. The book is clinically rather than scientifically based—and none the worse for this. It presents a solid, perhaps rather old-fashioned, but still sound, view of the subject. The numerous photographs, are, as before, in black and white but are mostly good.

Roxburgh must have had a steady market over the years but there is no hint, either in the preface or on the jacket, of whom the book was written for. The mildly didactic tone, the absence of references, and the detailed word pictures of common skin diseases, all suggest that it is either for medical students or for recently qualified general practitioners. Perhaps for both: as the student can swallow it all fairly quickly in a series of longish gulps before his exams, while it contains enough for the general practitioner to use it as a short reference book in his surgery. The book is stoutly bound and gives good value for its price.

J.A.SAVIN

The Year Book of Dermatology 1975. Edited by F.D.Malkinson and R.W.Pearson (1975) Chicago: Year Book Medical Publishers Inc. Price £11.30.

I have bought *The Year Book* for 15 years; they alter little in quality year by year—except to improve. Always outlasting the year's ephemera is the eagerly awaited feature article. The Porphyrias by L.C.Harber and D.R.Bickers is 1975's subject. The articles chosen for review follow, conveniently classified by subjects into 21 chapters. The selection of these articles always provokes comment from reviewers, some of them doubtless disappointed that their own literary brain child has been spurned. As is proper in this transatlantic publication, the bias is towards references from American writings. Some of the articles are merely anecdotal reports and their selection seems to be rather capricious, but obviously such a paper is far simpler to abstract and to comment upon. Sadly the longer, more complex or philosophical articles, perhaps minor masterpieces of writing and illustration, may find themselves relegated to the section now to be found at the end of each chapter, 'the following review articles are recommended to the reader'; an ever useful fount of references. I welcome the Editorial comments at the end of many of the abstracts. Perhaps some authors do have a genuine grumble that some of these are unfair or irrefutable or perhaps just trivial and one-up-ish, as on page 373 when the authors report 40 cases and the Editors add their own knowledge of another meagre 2. But, by and large, they transmute a potentially stodgy catalogue into a dermatologists' ideal bedside sleep-delaying volume. This edition is attractively produced and should tempt us all to improve ourselves by reading every word between the porphyry-pigmented covers of its special 75th Anniversary binding.

P.W.M.COPEMAN

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Review Article

The microbiology of dandruff

Some baffling problems lie in the border country between dermatology and cosmetology. They seem too trivial to whet the appetite of most clinical dermatologists and research is mainly driven by pressures from the market-place. Dandruff is a good example. Modern investigative techniques have, so far, only scratched the surface of dandruff, raising a cloud of conflicting ideas and reports.

It takes a determined clinician to obtain full-thickness excisional biopsies from forty-five subjects with dandruff (Ackerman & Kligman, 1969), but the results have been valuable. The disordered pattern of the stratum corneum found in dandruff, showing patchy parakeratosis, weakens the still current idea (Ebling & Rook, 1972) that dandruff is merely the normal shedding of stratum corneum hampered by the hairiness of the scalp. This idea was hard to support anyway, as dandruff is uncommon below the age of 5 (Ebling & Rook, 1972) although epidermal turnover must occur and hair is present. Clarification is still needed of the old clinical concepts of a dry and a greasy form of dandruff, and of the meanings of apparently contradictory terms such as seborrhoea sicca. But most modern studies, in defining their terms, regard dandruff as a non-inflammatory increased scaling which can be separated clinically even from minor degrees of seborrhoeic dermatitis. In addition, quantification of dandruff can be much improved in the laboratory by the use of corneocyte counts, using a haemocytometer. Kligman *et al.* (1974) claim that parakeratotic cells form up to 5% of the total in dandruff, but may be as much as 15–25% in seborrhoeic dermatitis.

The question of microbial involvement in dandruff is still unsettled. A hundred and two years ago Malassez (1874) appointed the yeast now named *Pityrosporum ovale* as chief suspect in the mystery of dandruff aetiology. Many since have tried to confirm or disprove the link between the two. In summary, most investigators, up to 1960, found no causal connection and today there is general agreement that *P. ovale* can be recovered from most, if not all, scalps, normal or otherwise. There are three reasons for reappraising the present situation. First, it has become clear that *P. ovale* can split sebum triglycerides to liberate irritant fatty acids, which could perhaps induce scaling. Secondly, there is an apparent conflict between the most recent work on the microbiology of dandruff (McGinley *et al.*, 1975) and certain other studies (VanderWyk & Hechemy, 1967; Roia & VanderWyk, 1969). Thirdly, improved schemes for classifying skin micro-organisms (Baird-Parker, 1963; Evans, 1968) should have assisted in identifying a dandruff flora, if such an entity existed. To the several sources of data on the normal scalp flora (Marples, 1965; Ackerman & Kligman, 1969; Roberts, 1969; Roia & VanderWyk, 1969; Noble & Somerville, 1974; McGinley *et al.*, 1975) we can add the results from an unpublished survey of dandruff-affected and normal scalps (Priestley, Hayday & Mitchell, unpublished).

With one exception, all agree that the aerobic bacterial flora of the scalp is dominated by coagulase-negative micrococci. Using Baird-Parker's (1963) classification, groups SII, M3 and M7 are most abundant, with smaller numbers of aerobic diphtheroids and bacillus species. When the criterion used is microscopic inspection rather than culture, *P. ovale* is always present (Roberts, 1969; McGinley *et al.*, 1975) and in large numbers. The anaerobe *Corynebacterium acnes* is also always present; McGinley *et al.* found that it accounted for about one-third of the microbes on the normal scalp, about 6% in dandruff, and only 1% in seborrhoeic dermatitis. They thought that the increased blood flow resulting from minor inflammation could account for the reduction in a strictly anaerobic organism. *C. acnes* varied inversely with the percentage of nucleated cells in the stratum corneum—an indication of increased epidermal turnover. There was also an inverse relationship between *C. acnes* and *P. ovale*.

The dissenting study is that of Roia & VanderWyk (1969), who found, remarkably, that only

57% of those with dandruff had any scalp bacteria at all (compared with 25% of those without dandruff). Their most prevalent bacterial species was *Bacillus subtilis* and micrococci were virtually absent. McGinley *et al.* were 'completely baffled' by these findings, since in their experience and that of most workers, aerobic cocci occur on all normal and diseased scalps.

Even with the refinement of Baird-Parker's scheme for sorting the cocci, there is no good evidence of any qualitative change in the flora of dandruff (McGinley *et al.*, 1975; Priestley *et al.* unpublished). A quantitative change seems more likely, if only because a scaling scalp provides a larger surface area for microbial growth. In fact, McGinley *et al.* (1975) could not confirm the earlier finding (Ackerman & Kligman, 1969) of an increase in the aerobic bacteria in dandruff, but they again found more *P. ovale*. The yeast made up almost 50% of the organisms on non-dandruff scalps, rising to 75% in dandruff and about 80% in seborrhoeic dermatitis.

The lipolytic activity of *P. ovale*, long suspected from its lipid dependence in culture (Benham, 1939), has now been demonstrated (Weary, 1970). In our experience (Priestley *et al.* unpublished) it is no more impressive than that of other skin micro-organisms and in fact Marples, Downing & Kligman (1972) found *C. acnes* to be by far the most potent source of free fatty acids on the scalp. The idea that *P. ovale* can lyse triglycerides to release fatty acids from sebum, some of which may irritate and produce scaling (Van Abbé, 1964) is essentially the microbial hypothesis in acne transferred to the scalp, and has the same merits and defects (Savin, 1972). At any rate strains of micrococci, diphtheroids and bacilli collected from subjects with dandruff did not hydrolyse more tryglyceride and Tween substrates than those isolated from normal scalps (Priestley *et al.* unpublished), and the possibility of a dandruff flora composed of more effectively lipolytic strains of these same organisms was not supported. A minority of sebum-free fatty acids irritate skin when liberally applied (Kellum, 1968) but the idea that the minute quantities naturally present can do so is more questionable. In our own work, using guinea-pig skin, scaling was only induced by completely unphysiological loads of C_{12} saturated or C_{16}–C_{18} unsaturated acids applied to the skin (Priestley *et al.* unpublished).

VanderWyk & Hechemy (1967) claimed that reduction of yeasts with nystatin controlled dandruff more effectively than did suppression of bacteria with antibiotics. Their complicated paper is greatly weakened by failure to test the data statistically, and the significance of the findings is open to doubt. Another problem comes from reliance on the experimental subjects applying different lotions to each side of their own scalps three times daily for several weeks. The scalp is not a suitable area for this type of trial due to drug translocation effects (Marples & Kligman, 1973). Gosse & VanderWyk (1969) tried to eliminate the total scalp flora of eleven subjects by applying a mixture of neomycin and nystatin, and then recolonizing with a nystatin-resistant strain of *P. ovale* while the mixture was still being applied. Although the application of *Pityrosporum* coincided with increased scaling, the lack of statistical analysis, coupled with the absence of a control group to identify seasonal or other long-term trends in scaling (Van Abbé, 1964; Kligman *et al.*, 1974), make interpretation of this elaborate work very difficult. In other hands (Ackerman & Kligman, 1969; McGinley *et al.*, 1975) topical antibiotic agents chosen to suppress either bacteria or yeasts had little effect on scaling.

If these agents can control micro-organisms but not dandruff, what is the action of agents which do control dandruff? Normal non-medicated shampoos seem to have a mildly beneficial effect (Van Abbé, 1964; Alexander, 1967; Kligman *et al.*, 1974), perhaps simply by washing off obvious loose scales, but there is now experimental data to support claims that shampoos with selenium disulphide or zinc omadine (zinc pirithione) are particularly effective (Kligman *et al.*, 1974). These compounds have a wide range of antibacterial and antifungal action (Brauer, Opdyke & Burnett, 1966; Brotherton, 1967) but there are intriguing hints of other actions as well. Plewig & Kligman (1970) indicate that selenium disulphide is antimitotic, acting on dandruff by reducing the increased epidermal turnover revealed by tritiated thymidine autoradiography. Zinc omadine could have a similar antimetabolic action on microbes and epidermis alike. The link between scaling conditions and zinc metabolism, well known in domestic and laboratory animals (Vallee, 1959) and dramatically shown in the treatment of acrodermatitis enteropathica (Barnes & Moynahan, 1973), raises the idea that the zinc may be remedying some local deficiency in the scalp. Perhaps monitoring of both scalp scaling and scalp microflora in patients on systemic antimitotic treatments might hold the key to this problem by separating the microbial and epidermopoietic elements, and finally exorcize the ghost of Malassez.

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REFERENCES

ACKERMAN, A.B. & KLIGMAN, A.M. (1969) Some observations on dandruff. *Journal of the Society of Cosmetic Chemists*, **20**, 81.

ALEXANDER, S. (1967) Do shampoos affect dandruff? *British Journal of Dermatology*, **79**, 92.

BAIRD-PARKER, A.C. (1963) A classification of micrococci and staphylococci based on physiological and biochemical tests. *Journal of General Microbiology*, **30**, 409.

BARNES, P.M. & MOYNAHAN, E.J. (1973) Zinc deficiency in acrodermatitis enteropathica; multiple dietary intolerance with synthetic diet. *Proceedings of the Royal Society of Medicine*, **66**, 327.

BENHAM, R.W. (1939) The cultural characteristics of *Pityrosporum ovale*—a lipophylic fungus. *Journal of Investigative Dermatology*, **2**, 187.

BRAUER, E.W., OPDYKE, D.L. & BURNETT, C.M. (1966) The anti-seborrheic properties of zinc pyrithione (zinc pyridine-2-thiol-1-oxide) in a cream vehicle. *Journal of Investigative Dermatology*, **47**, 174

BROTHERTON, J. (1967) The sulphur metabolism of *Pityrosporum ovale* and its inhibition by selenium compounds. *Journal of General Microbiology*, **49**, 393.

EBLING, F.J. & ROOK, A.J. (1972) Pityriasis capitis. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1639. Blackwell Scientific Publications, Oxford.

EVANS, N.M. (1968) The classification of aerobic diphtheroids from human skin. *British Journal of Dermatology*, **80**, 81.

GOSSE, R.M. & VANDERWYK, R.W. (1969) The relationship of a Nystatin-resistant strain of *Pityrosporum ovale* to dandruff. *Journal of the Society of Cosmetic Chemists*, **20**, 603.

KELLUM, R.E. (1968) Acne vulgaris. Studies in pathogenesis: relative irritancy of free fatty acids from C2 to C16. *Archives of Dermatology*, **97**, 722.

KLIGMAN, A.M., MARPLES, R.R., LANTIS, L.R. & MCGINLEY, K.J. (1974) Appraisal of efficacy of antidandruff formulations. *Journal of the Society of Cosmetic Chemists*, **25**, 73.

MALASSEZ, L. (1874) Note sur le champignon de la pelade. *Archives of Physiology and Normal Pathology II*, **1**, 203.

MARPLES, M.J. (1965) *The Ecology of the Skin*. Charles C.Thomas, Springfield, Illinois.

MARPLES, R.R., DOWNING, D.T. & KLIGMAN, A.M. (1972) Control of free fatty acids in human surface lipids by *C. acnes*. *Journal of Investigative Dermatology*, **54**, 434.

MARPLES, R.R. & KLIGMAN, A.M. (1973) Limitations of paired comparisons of topical drugs. *British Journal of Dermatology*, **88**, 61.

MCGINLEY, K.J., LEYDEN, J.J., MARPLES, R.R. & KLIGMAN, A.M. (1975) Quantitative microbiology of the scalp in non-dandruff, dandruff, and seborrheic dermatitis. *Journal of Investigative Dermatology*, **64**, 401.

NOBLE, W.C. & SOMERVILLE, D.A. (1974) *Microbiology of Human Skin*, p. 268. Saunders, London.

PLEWIG, G. & KLIGMAN, A.M. (1970) Zellkinetische untersuchungen bei Kopfschuppenerkrankung (Pityriasis simplex capillitii). *Archiv für klinische und experimentelle Dermatologie*, **236**, 406.

ROBERTS, S.O.B. (1969) The mycology of the clinically normal scalp. *British Journal of Dermatology*, **81**, 626.

ROIA, F.C. & VANDERWYK, R.W. (1969) Resident microbial flora of the human scalp and its relationship to dandruff. *Journal of the Society of Cosmetic Chemists*, **20**, 113.

SAVIN, J.A. (1972) The bacteriology of acne. *British Journal of Dermatology*, **86**, Supplement 8, 3.

VALLEE, B.L. (1959) Biochemistry, physiology and pathology of zinc. *Physiological Reviews*, **39**, 443.

VAN ABBÉ, N.J. (1964) The investigation of dandruff. *Journal of the Society of Cosmetic Chemists*, **15**, 609.

VANDERWYK, R.W. & HECHEMY, K.E. (1967) A comparison of the bacterial and yeast flora of the human scalp and their effect upon dandruff production. *Journal of the Society of Cosmetic Chemists*, **18**, 629.

WEARY, P.E. (1970) Comedonic potential of the lipid extract of *Pityrosporum ovale*. *Archives of Dermatology*, **102**, 84.

British Journal of Dermatology (1976) **94**, 472.

News and Notices

Joint Advanced Course in Paediatric Dermatology: Institutes of Dermatology and Child Health (University of London)

This course is open to dermatological and paediatric consultants and senior registrars. It will be held from 20 to 24 September 1976 at the Institute of Child Health, 30 Guilford Street, London WC1. It is expected that the course will be recognized under the Advanced Postgraduate Training Scheme of the DHSS. Requests for further details and applications should be sent to the Dean, Institute of Child Health, not later than 30 April, 1976.

Second Henry E.Michelson Memorial Lecture

Richard B.Stoughton, M.D., Head of the Division of Dermatology at the Scripps Clinic and Research Foundation, La Jolla, California, will deliver the Memorial Lecture at the Second Henry E.Michelson Clinical and Scientific Seminar of the Minnesota Dermatological Society to be held on Friday, 21 May 1976, in Minneapolis, Minnesota. Presentations of dermatological interest and Dr Stoughton's lecture will be given in the morning; clinical case presentations and discussions will follow in the afternoon. Reservations and further information may be obtained from: Dr William C.Gentry, Jr., Box 98, University Hospitals, Minneapolis, Minnesota 55455, U.S.A.

APPOINTMENTS

University College Hospital

Dr Jeremy John Heming Gilkes has been appointed Consultant Dermatologist to University College Hospital, London, and Senior Lecturer in Dermatology to University College Hospital Medical School. Dr Gilkes graduated M.B., B.S.(London) 1964 and became M.R.C.P.(U.K.) in 1970, proceeding M.D. (London) in 1975. He became a medical cadet with the R.A.M.C. whilst a student at St Bartholomew's Hospital. During his service with the R.A.M.C. (1965–70) he served in the Far East and also as a medical specialist at Queen Alexandra Hospital, Millbank, where he obtained the M.R.C.P. His training in Dermatology was at Guy's Hospital as a registrar (1970–2) and St Bartholomew's Hospital as a Senior Registrar (1972–5). His main research interests and publications have been concerned with the melanotrophic peptides in human plasma, he has also written several papers on subjects of clinical interest.

Croydon Area and Merton, Sutton and Wandsworth Area

Dr Kurwa has been appointed Consultant Dermatologist to the Croydon Area and Merton, Sutton and Wandsworth Area. Dr Kurwa qualified M.B., B.S., from the University of Bombay in 1958 and became M.R.C.P.(Edin.) in 1965. He came to England in 1958 and trained at various hospitals at London, Plymouth and Norfolk before becoming Senior Registrar in Dermatology at Cardiff Royal Infirmary and later at the United Oxford Hospitals. He has written a number of papers of clinical interest.

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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References 1. Whitefield, M. and McKenzie, A. W., Brit. J. Derm. (1975), **92,** 585. 2. Fredriksson, T. and Gip, L., Dermatology Digest. (1976) **Jan.** 27. 3. Barry, B. W. and Woodford, R., Brit J. Derm. Accepted for publication.

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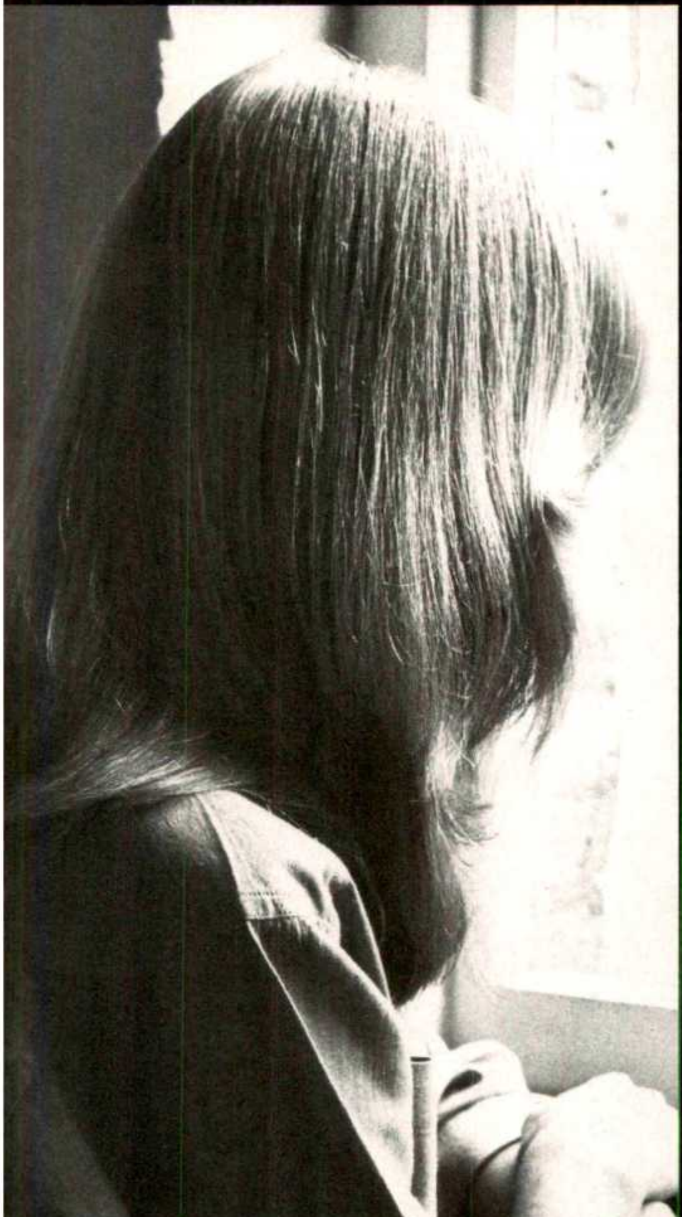

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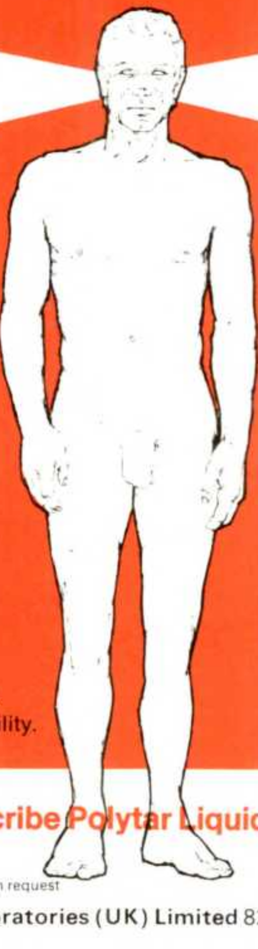

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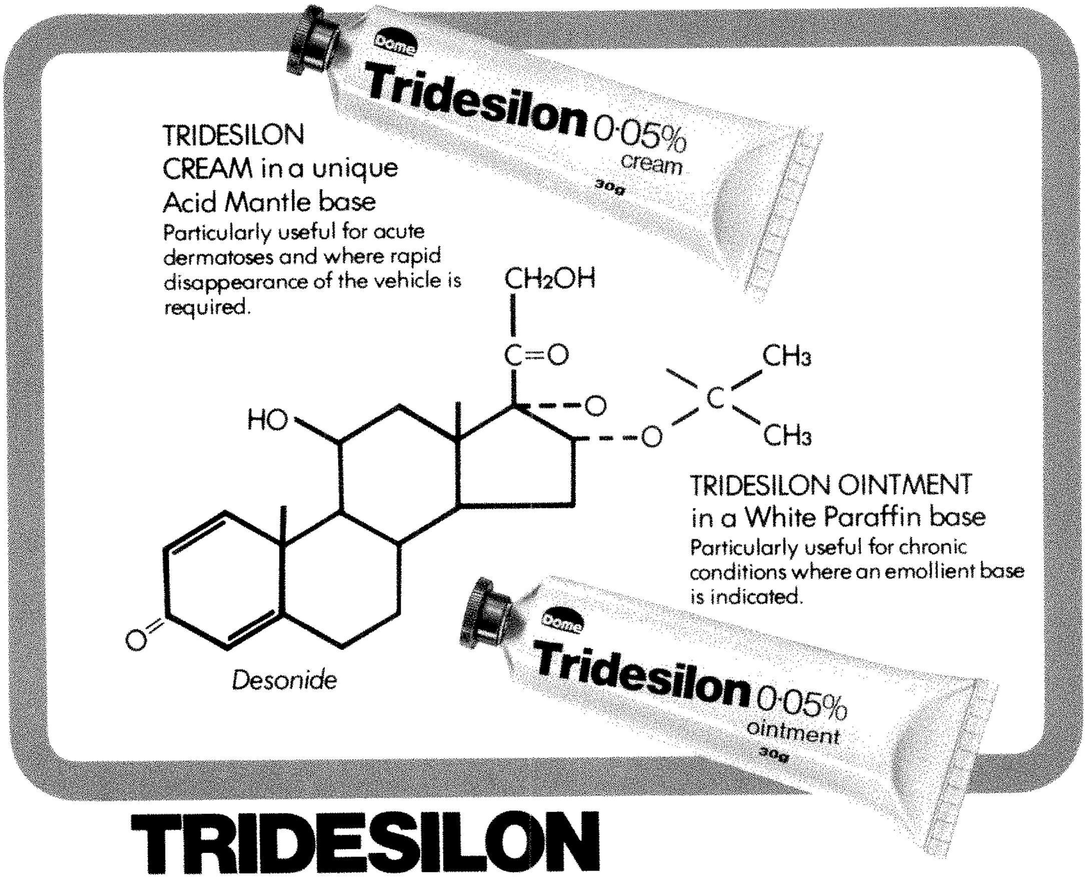

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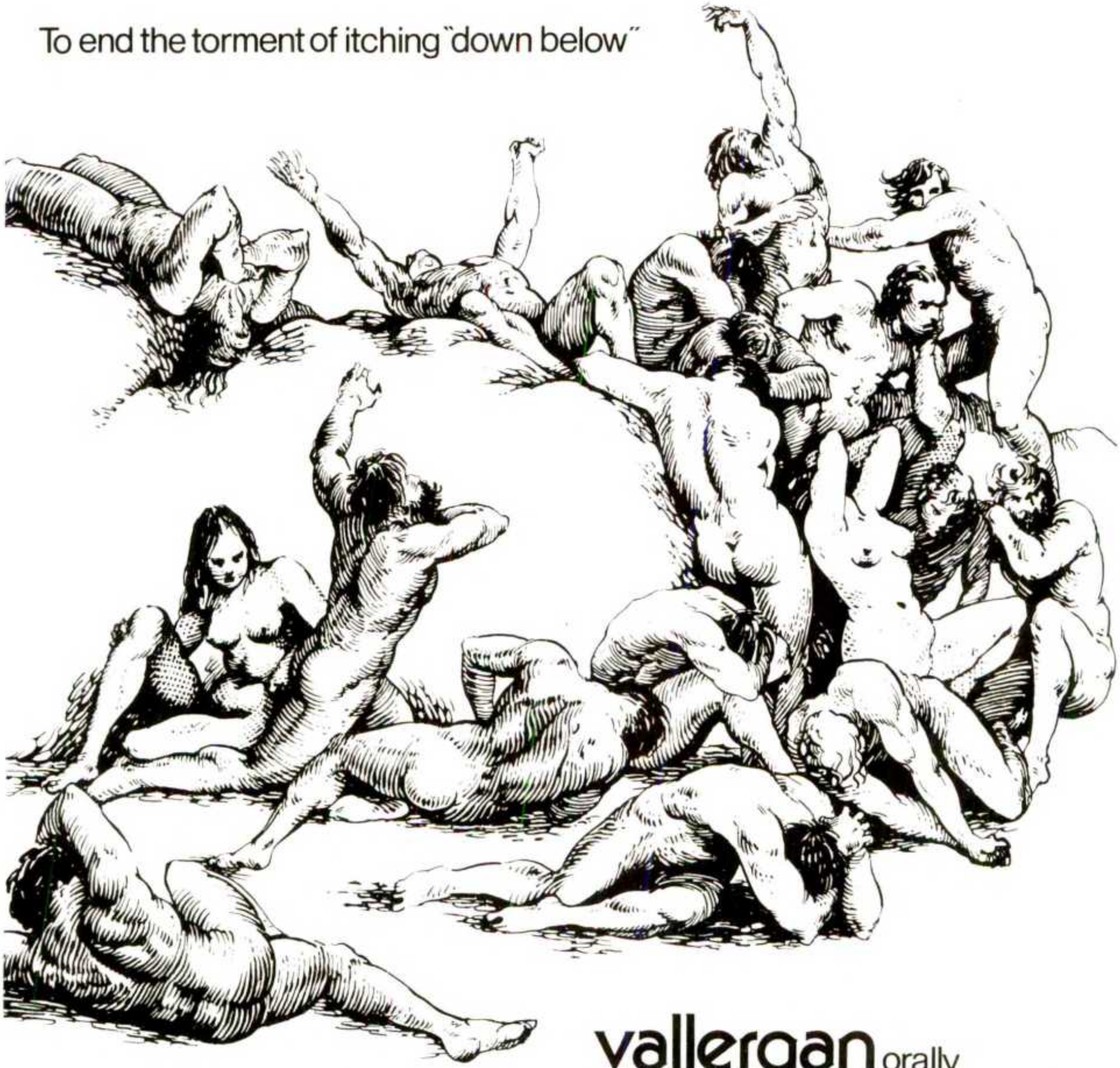

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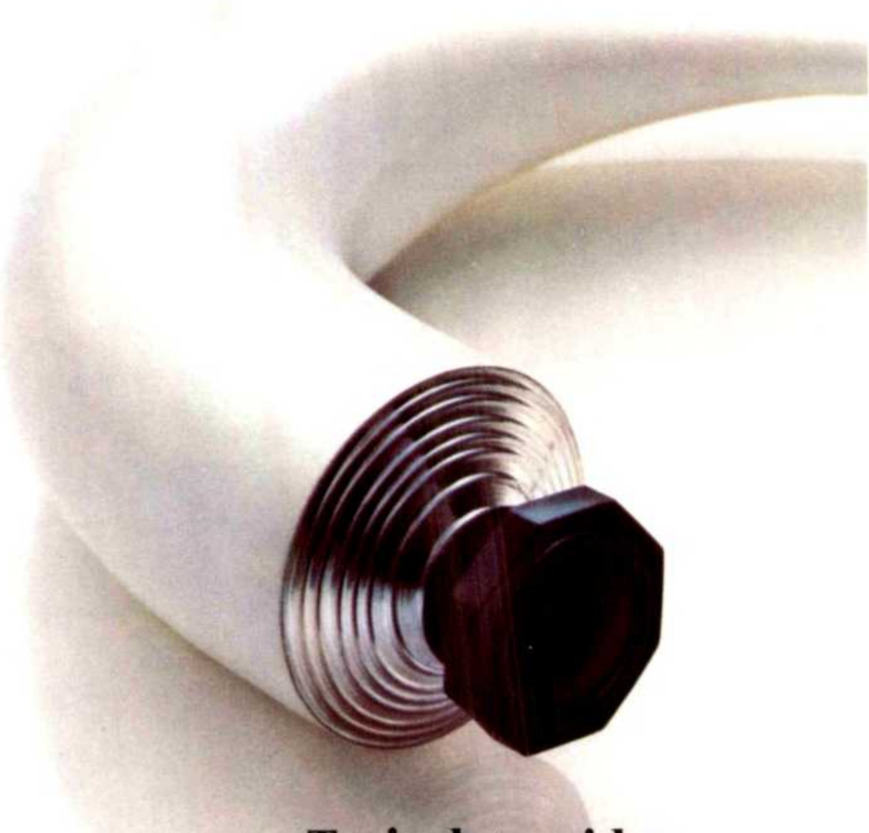

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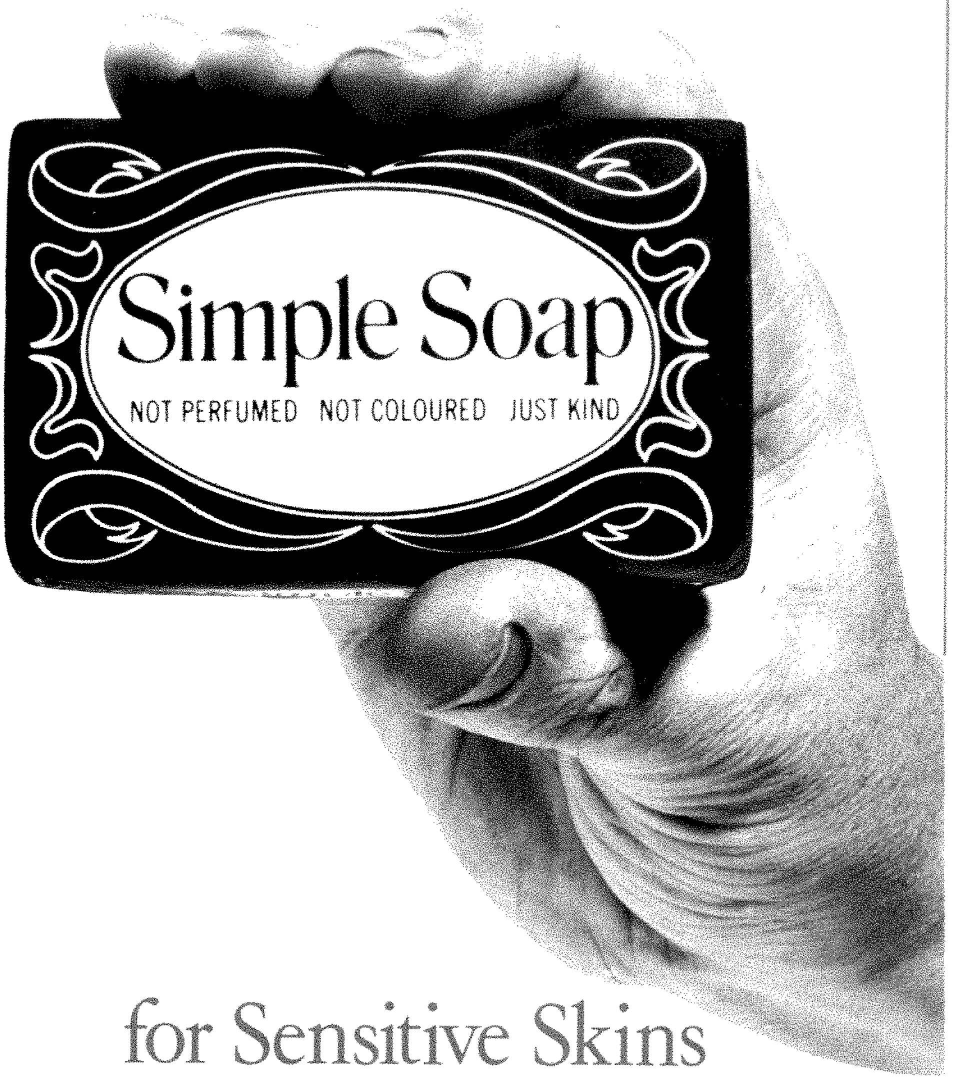

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Practitioner*, (1974), **213, 864.

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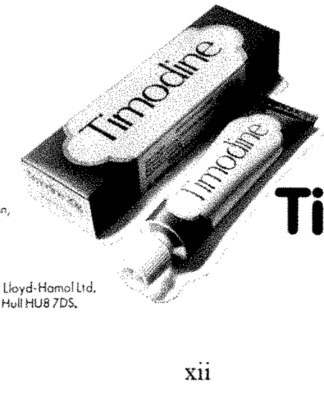

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1. Bunney, Mary H. (1971), Practitioner, **207**, 197.
2. Pegum, J. S. (1972), Practitioner, **209**, 453.
3. Bunney, Mary H. (1974), Prescribers Journal, **14**, 118.

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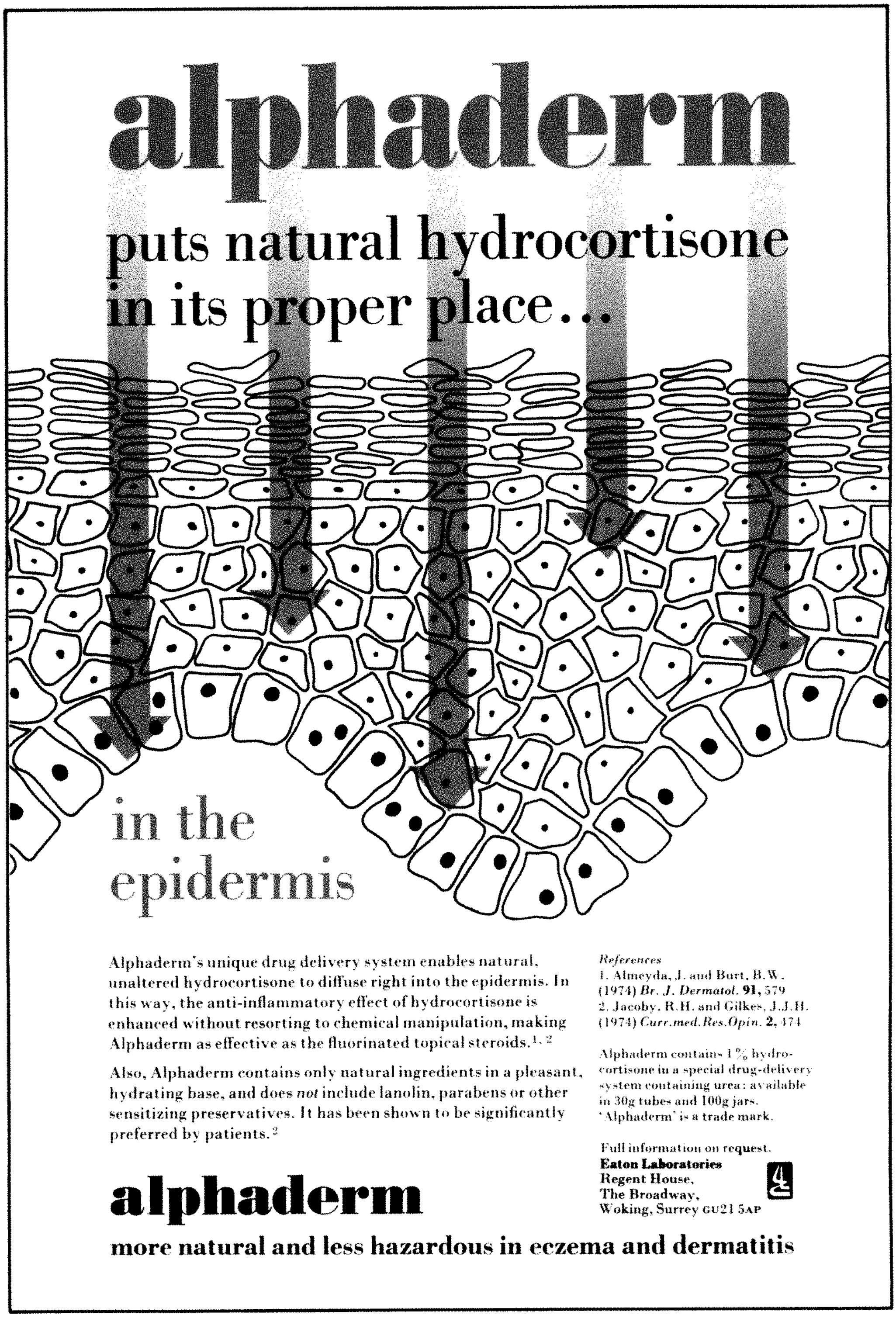

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1. Almeyda, J. and Burt, B.W. (1974) *Br. J. Dermatol.* **91,** 579
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British Journal of Dermatology (1976) **94**, 473.

Clinical and Laboratory Investigations

The pathology of lymphangioma circumscriptum

I.W.WHIMSTER

Department of Dermatology, St Thomas' Hospital and Medical School, London SE1

Accepted for publication 30 August 1975

SUMMARY

A study of lymphangioma circumscriptum has been carried out over a number of years in an attempt to understand its nature and pathogenesis, with a view to improving treatment. It is postulated that the lesion consists essentially of a collection of large muscular-coated lymphatic cisterns, lying deep in the subcutaneous plane and communicating via dilated dermal lymphatics with the superficial vesicles. It seems that these cisterns do not communicate directly, if at all, with the general lymphatic system but represent a sequestrated part of it. They have been shown by cannulation to pulsate at a slow steady rate producing a rise and fall of pressure within them. It is believed that the vesicles are saccular dilatations of superficial lymphatics, secondary to raised pressure transmitted from the pulsating cisterns beneath. It is suggested that it may be possible to treat these lesions more successfully and with better cosmetic results by excising the subcutaneous cisterns and leaving the overlying skin intact.

Lymphangioma circumscriptum (L.C.) is characterized clinically by the presence within a circumscribed area of skin of vesicles, mostly filled with clear colourless fluid but occasionally tinged with varying quantities of blood, the colour then ranging from pink, through red to black. The clinical aspects of the condition were reviewed by Peachey, Lim & Whimster in 1970. The present article is concerned entirely with the type of lesion defined by these authors as the 'classical' form (Fig. 1).

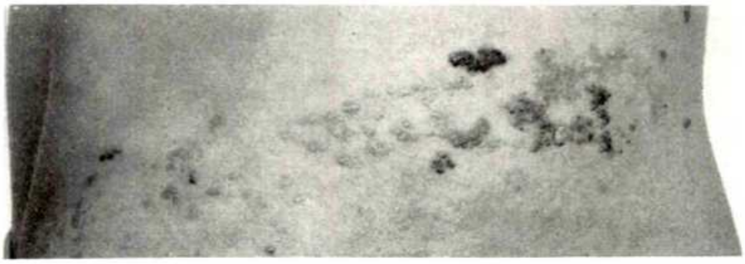

FIGURE 1. A circumscribed group of L.C. vesicles, ranging from colourless to dark red, just above the left iliac crest of a girl of 14 years. They had been present and increasing in numbers since the age of 6 years. Beneath the involved skin there was a deep diffuse subcutaneous swelling.

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* Extension of a paper presented at the B.A.D. Meeting, London, July 1974.

The condition is usually present at birth or develops soon afterwards, commonly becoming more intense throughout childhood and persisting indefinitely. It is a nuisance to the patient, not only because of the cosmetic disfigurement caused by the presence of the vesicles but also because of their tendency to rupture and leak fluid which stains clothing.

While being well known and easily recognized by its clinical manifestations there were two features about the condition which seemed to call for further investigation. First, its pathogenesis was unknown and, secondly, it was apparent that present methods of treatment were profoundly unsatis-

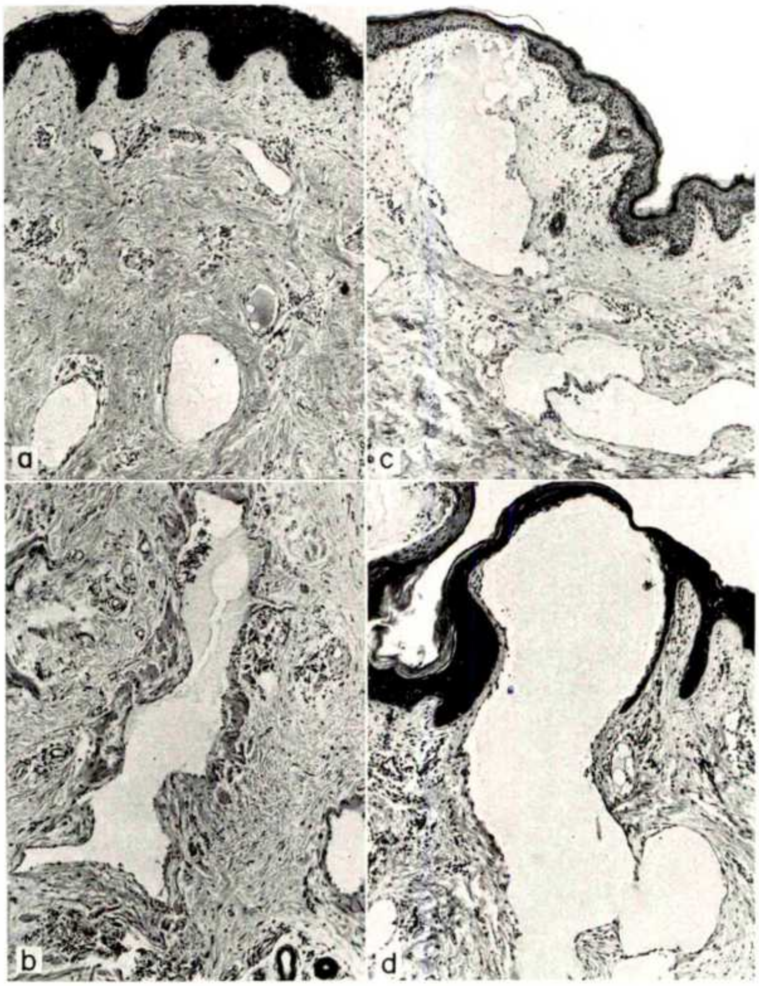


FIGURE 2 (a) Skin from within an area of L.C. but between vesicles. In the upper dermis there are grossly dilated lymphatic vessels orientated mainly in the horizontal plane.

(b) Enlarged and dilated muscle-coated lymphatic running vertically in the lower dermis beneath an area of vesicles.

(c) An early stage in vesicle formation. The dilated vessels in the photograph can be seen in serial sections to be interconnected. Beneath the epidermis is a very dilated thin walled lymphatic arising from the horizontal vessels as a blind-ended sac.

(d) Fully formed L.C. vesicle occupying a whole papilla and arising like a balloon from a horizontal vessel lying just below the papillary layer.

factory. It was felt that if the pathology of the condition were better understood this might in turn lead to improved methods of therapy.

This article is an attempt to set out the progressive series of observations which have been made over a number of years of the morbid anatomy, the histology and the natural history of the condition, together with the sequence of conclusions, hypotheses and further investigations arising out of them. The study has been based mainly on thirty cases from which whole surgical excision specimens were available for dissection and histological examination by the author, backed up by many further cases from which less complete material was available.

Observations

(1) However large or small the area of skin involved in L.C. may be, it is as the name implies a circumscribed one. The arrangement of herpes zoster lesions within a characteristically *circumscribed* area of skin, as opposed to the *diffuse scattering* of varicella lesions, led to the recognition of the reason for this grouping and to the common link between all the lesions of a group: a posterior root ganglion. It was felt that the circumscribed grouping of L.C. vesicles must have a similar significance, in that it indicated the presence of some underlying link between all the individual and apparently separate vesicles within the circumscribed area.

(2) In L.C. the dermis immediately beneath the papillary layer contains dilated thin-walled lymphatics mainly orientated horizontally, and deep to these are others, some with thicker walls, mainly running vertically (Fig. 2a, b).

(3) The vesicles of L.C. are saccular dilatations bulging out from thin-walled lymphatics and projecting upwards to occupy the papillae only when sufficiently distended. So far as I have personally been able to convince myself there are no lymphatics in normal dermal papillae (Fig. 2c, d).

(4) The vesicles are often not present at birth, and even when some are, others certainly arise later. This suggests that the dilatation of the saccular vessels is not due to any intrinsic fault of the normal

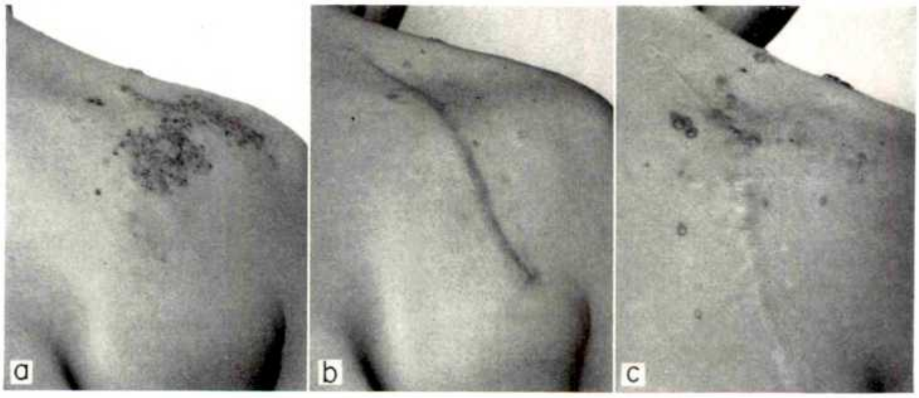


FIGURE 3. (a) A girl aged 9 years with a group of L.C. vesicles on the right shoulder. Two vesicles were present at birth and their number had increased subsequently.

(b) The same patient 6 months after excision of almost all the affected skin together with subcutaneous tissue to a total depth of 10 mm. There were large abnormal lymphatics transected at the base of the excision specimen. At this post-operative stage there are already a few new vesicles forming alongside the scar.

(c) The same patient 2 years later. There are now almost as many newly formed vesicles in the area as there were in the original lesion.

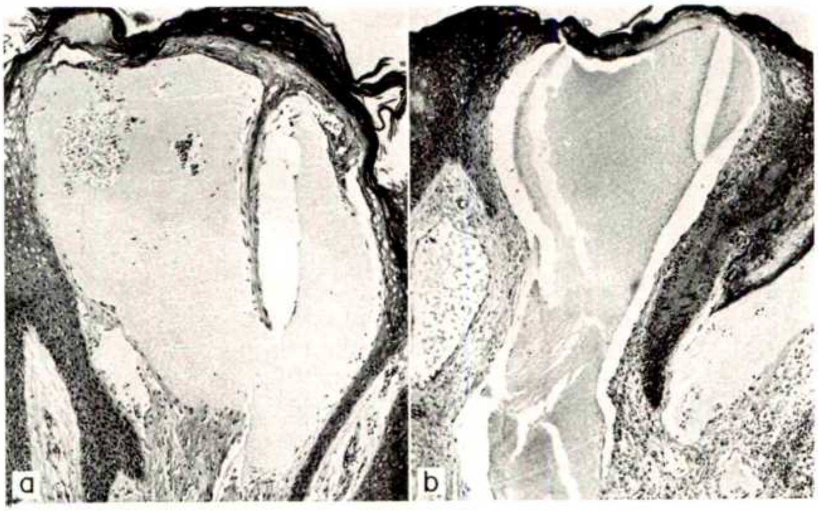


FIGURE 4. (a) Female aged 18 years. A vesicle from an area of typical L.C. The affected skin was excised and the wound repaired with a full thickness pedicle graft. Subsequently the graft developed abundant new vesicles.
(b) The same patient as in (a). A vesicle in the graft about 18 months after operation.

dermal lymphatics of the area concerned but that the fault, whatever its nature, is an acquired one, affecting vessels which either appeared normal previously or were not in existence at that time.

(5) After excision of lymphangiomatous skin, with the full thickness of dermis and including all the vesicles present, 'recurrence' of vesicles (that is to say the formation of new vesicles) is the rule, whether the wound has been sutured or grafted. This phenomenon indicates that skin whose superficial lymphatics have previously behaved normally can be made to develop saccular ones as a result of its coming to occupy the site of the original lesion. Fig. 3 (a–c) shows new vesicles developing in previously normal skin at the margins of an excision scar and Fig. 4 (a, b) in a full thickness graft.

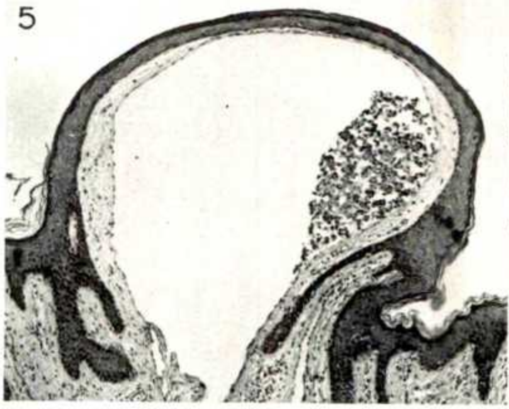
Conclusion

The conclusion reached from this basic set of observations is that in L.C. there is something wrong with the lymphatic system of the *site* and not just with the dilated vessels in the skin, nor indeed with the skin itself. It seems that the primary fault must lie somewhere at a deeper plane, beneath the skin, and that one of the effects of this deep abnormality is to cause saccular dilatation of superficial lymphatics in a circumscribed area of overlying skin.

Observations

(6) Similar vesicles formed by saccular dilatations of superficial lymphatics sometimes occur in association with lymphoedema of a limb or scrotum due to acquired lymphatic obstruction. This suggests that such vesicles are the result of raised intralymphatic pressure (Fig. 5).

(7) In the conventional excision specimen of L.C., consisting of full thickness dermis and a partial layer of subcutaneous fat, there are always abnormally large lymphatics, some with muscle coats, to be seen transected at the lower margin of the specimen. These can be traced in serial sections to communicate with the more superficial thin-walled dermal vessels and via them with the vesicles (Figs 6 and 7).



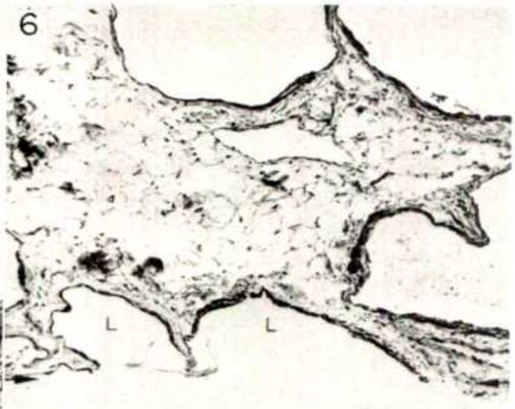


FIGURE 5. Male aged 53 years. Subepidermal lymphatic vesicle from the scrotum of a patient with severe lymphoedema due to acquired lymphatic obstruction. The appearance of the vesicle is indistinguishable from one of L.C.

FIGURE 6. Inadequate depth of excision. Section through the lower margin of an 'orthodox' excision specimen of L.C. The plane of excision (arrowed) was 15–20 mm below the skin surface and, as can be seen, grossly dilated muscle-coated lymphatics (L) in the subcutis have been transected at operation and their deeper parts left in the patient. As in the case shown in Fig. 3 new vesicles were developing around the scar a year later.

Conclusion

The conclusion drawn from these two observations is that the large vessels in L.C., running mainly vertically through the dermis, are transmitting raised pressure to the superficial vesicles. Furthermore it seems that this raised pressure must come from some peculiarly localized and confined source rather than from the lymphatic system proper; otherwise there would be lymphoedema, at least of the

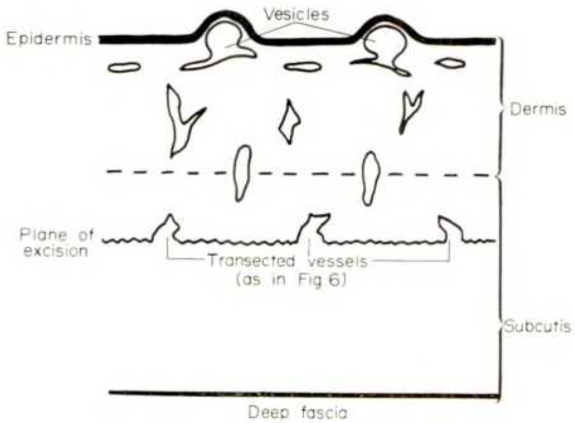


FIGURE 7. Diagram to illustrate the histological findings in L.C. specimens excised with only part of the thickness of subcutaneous fat. Included in such specimens are the subepidermal vesicles and the superficial dilated vessels from which they arise, together with large dilated vessels in the dermis and subcutis. But at the deep margin of the excision these vessels are cut across and their proximal parts remain in the bed of the wound.

area of skin containing the vesicles, if not of the whole of the limb or of the region of the trunk concerned. And L.C. is in my experience never accompanied by oedema.

Observation

(8) It is not unusual to find histiocytes (macrophages) and even giant cells lying free within the lumen of the dilated dermal lymphatics in L.C. These cells may occur singly or in quite large clumps. Frequently their cytoplasm is vacuolated and on occasions they can be seen to have ingested red blood cells or to contain iron pigment (Fig. 8a–c).

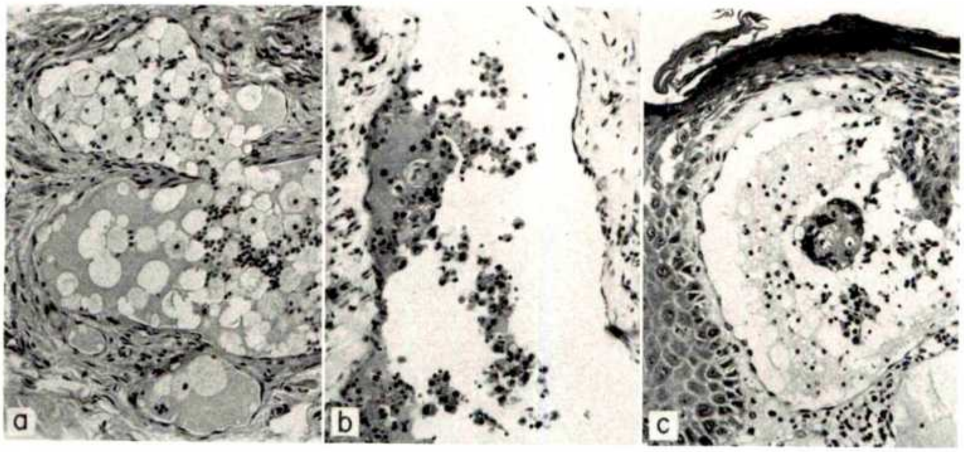


FIGURE 8. (a) Dilated L.C. vessele containing large foamy histiocytes and some red cells.
(b) Dilated L.C vessel containing histiocytes laden with iron pigment.
(c) Vesicle from a superficially inflamed area of L.C. The lumen contains a giant cell as well as lymphocytes and histiocytes.

Conclusion

The presence of such phagocytic cells lying free within the lumen of a vessel strongly suggests that there is not a normal flow of fluid within the vessel concerned. It suggests that there is no true circulation in or drainage from these vessels and that they may form part of a closed system in which the fluid is almost static.

Observation

(9) As stated earlier it is common for L.C. vesicles to have their clear, colourless fluid contaminated by varying quantities of red cells. This mixture of blood and lymphangioma fluid occurs predominantly at the most peripheral part of the lesion, in the vesicles and the thin-walled dilated lymphatics just beneath them. It seems that minor haemangiolymphatic connections must occur now and again, perhaps caused by mild trauma, in the region where blood capillaries are lying immediately adjacent to dilated lymphatics and indeed often stretched by them (Fig. 9a, b). Vesicles which have become pink or red due to blood contamination remain so for weeks before clearing. And vesicles which have become filled with pure blood turn black, dry up and fall off. Histologically such vesicles are seen to be entirely filled by packed red cells (Fig. 9c, d).

Conclusion

These observations concerning the contamination of vesicle fluid by blood further support the sug-

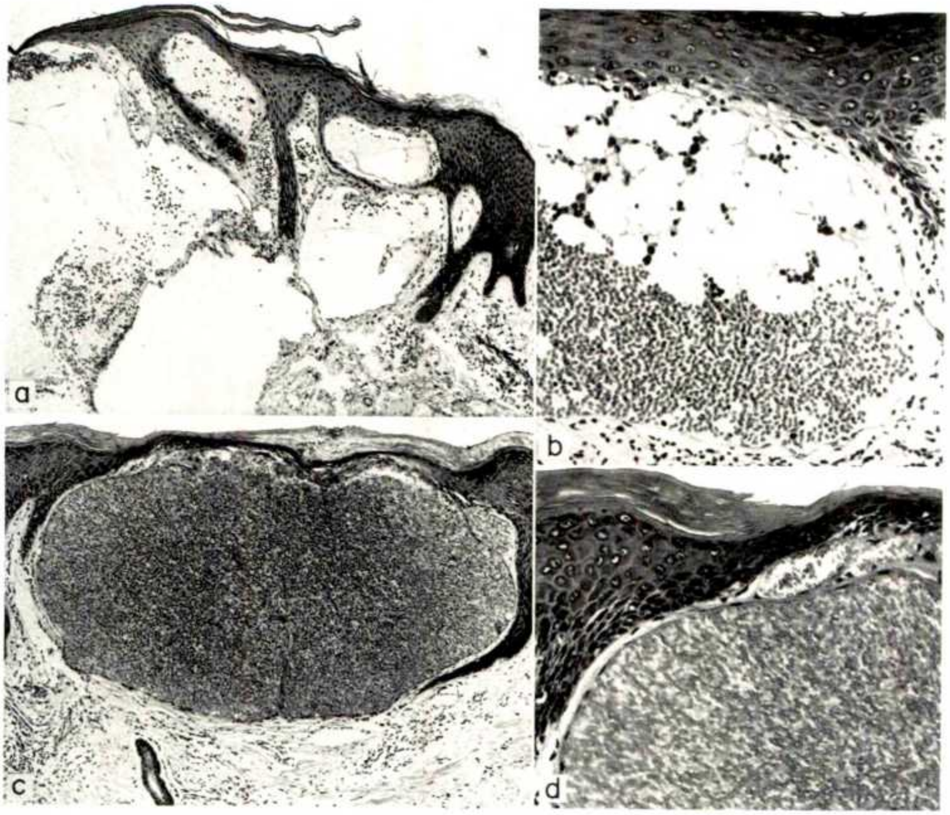


FIGURE 9. (a) L.C. vesicles with a small number of red cells in their fluid. These vesicles were macroscopically pink.

(b) L.C. vesicle containing about 50% of red cells in its fluid. There are also a few histiocytes present. This vesicle was macroscopically red.

(c, d) L.C. vesicle whose lumen is filled with packed red cells. This vesicle, which was macroscopically blackish red, would shortly have dried up and fallen off as a little black crust. The epidermis overlying it is already becoming necrotic.

gestion that there is no true circulation in or drainage from the vesicles. If there were, red cells entering vesicles would be rapidly flushed away and not remain until phagocytosed by histiocytes or congealed into a solid mass.

Observation

(10) As a result of these preliminary observations, and the conclusions which they suggested, it was decided to encourage the surgeons to excise L.C. lesions more deeply; in fact right down to the level of the deep fascia. When such deep excision specimens came to hand and were dissected it was found that the large dermal lymphatics could be followed down through the subcutaneous fat and into one or more large cisterns lying just above the deep fascia. These very large spaces, often several centimetres in diameter, do not have the form of linear vessels but are cystic, often with trabeculated linings and always with abundant smooth muscle in their walls. Individual cisterns are commonly multilocular and beneath most areas of L.C. there is more than one cistern present (Fig. 10a–c). When multiple the cisterns do not interconnect. Numerous lymphatic vessels lead out of these cisterns

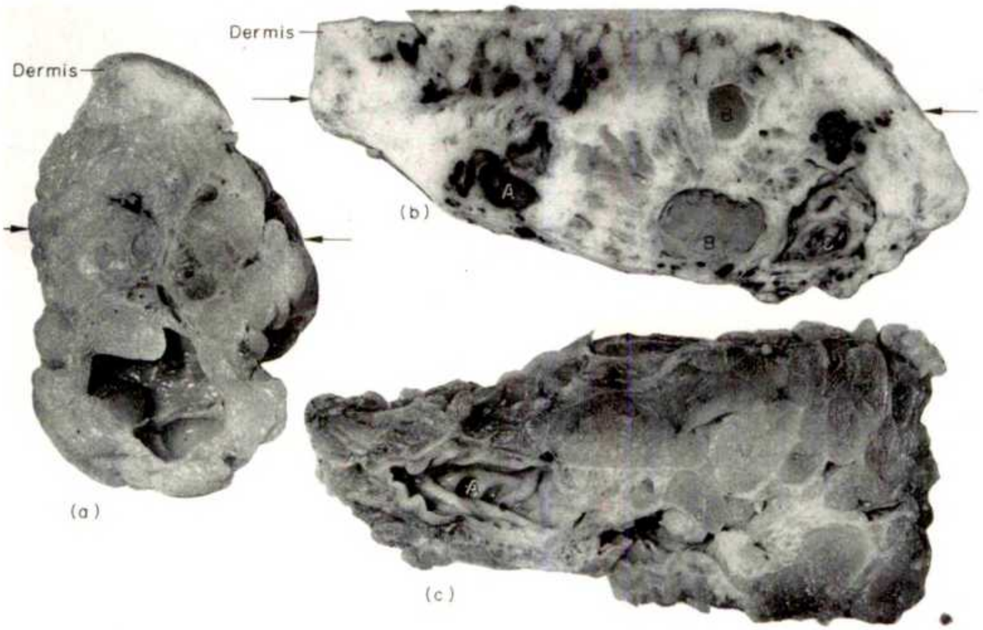


FIGURE 10. (a) A slice through a deep exision specimen of L.C. The depth of the subcutaneous tissue excised was 40 mm and in its deepest part is a typical cistern, multilocular and with a trabeculated wall. (The arrows indicate the plane of 'conventional' excision.)

(b) Deep excision specimen from another case. The depth of tissue removed was 55 mm. Several separate cisterns were present, three of them shown in this photograph. B is still filled by lymphangioma fluid, clotted by fixation, while A and C have had their contents removed. Note the strongly trabeculated wall of C. (The arrows indicate the plane of 'conventional' excision.)

(c) Subcutaneous tissue 50 mm in depth removed from beneath the L.C. shown in Fig. 1. Several cisterns were present and parts of two of them, each about 30 mm diameter, are shown. Note the very thick (2 mm) trabeculated wall of A. The histology of this cistern is shown in Fig. 11a.

and run up through the subcutaneous fat to communicate with the large dermal lymphatics, and thence to the surface of the skin. There is no sign of any vessels leaving the deep aspect of these cisterns to join the main lymphatics which lie at that plane and drain centrally (Fig. 11a, b).

Conclusion

These findings seemed to support the growing belief that the abnormal vessels of L.C. may form part of a closed system. The presence of a well-developed muscle coat around the cisterns suggested that this muscle must *do* something; either exercise a continous tone or else contract intermittently and pulsate like the peripheral lymph hearts of lower vertebrates, or both.

HYPOTHESIS

The hypothesis arrived at by the dissection and histological examination of a series of full depth excision specimens of L.C. was thus as follows. The complete lesion consists of an apparently closed system of lymphatic vessels, arising from large muscle-coated cisterns which lie just above the deep fascia and exert a continuous or intermittent pressure on their contained fluid, which is transmitted

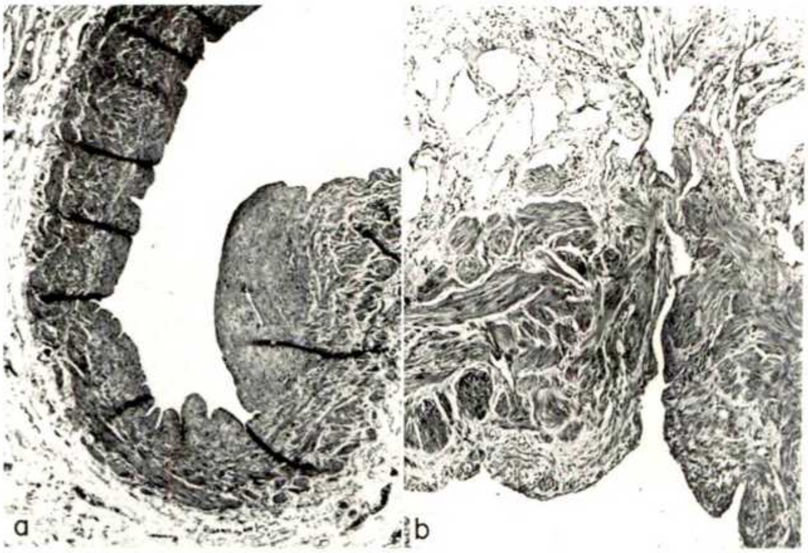


FIGURE 11. (a) Low power view of part of the large cistern A shown in Fig. 10c. It was 30 mm in diameter and its muscular wall 2 mm thick.

(b) Higher magnification of part of the muscular wall of a cistern from a different case, showing one of the vessels which traverse the wall and expand outside it into leashes of dilated lymphatics.

through the skin via large dilated lymphatics to the surface, where saccular dilatation of subepidermal lymphatics occurs, giving rise to the clinically visible vesicles (Fig. 12).

Observations made in vivo

(11) Lymphangiography has been carried out in the majority of cases in this series. In all the cases so studied normal lymphangiograms of the related limb have been obtained and in no case has there been any filling of the cisterns or other parts of the lymphangioma. There is, it seems, no retrograde connection between the normal main lymphatics and the cisterns.

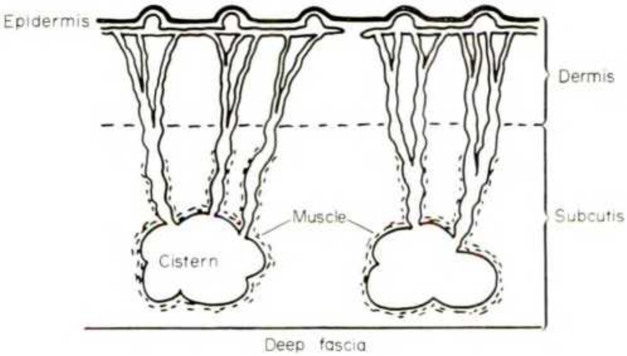


FIGURE 12. Diagram to illustrate the hypothetical reconstruction of a complete L.C. lesion. In fact the abnormal lymphatics running from the cisterns to the skin surface are much more complex in their layout than portrayed here. They adopt a meandering course with frequent anastomoses and branchings and many have varicosities along their course.

(12) On a number of occasions dye or contrast medium has been injected into the cisterns at operation and has been observed not to drain away into any normal lymphatics. The cisterns appear to be truly isolated from the main lymphatics of the region.

(13) It has been confirmed that when multiple the cisterns do not intercommunicate. If one is punctured during operation and its contained fluid leaks out, the others do not become deflated. And dye injected into one cistern does not appear in neighbouring ones.

(14) When the lesion is being undercut at the plane of the deep fascia it has been observed that the cisterns do not leak or collapse, and by the time such specimens arrive in the laboratory they are still full of fluid (Fig. 10b).

(15) At no time has the operating surgeon in the course of undercutting the cisterns been able to detect any connections at all between them and the normal lymphatics of the region.

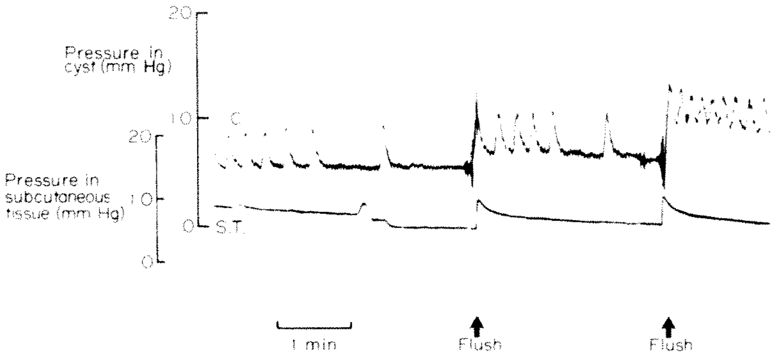


FIGURE 13. The upper tracing is from a cannula inserted into an L.C. cistern. It shows fairly regular pulsations, initially at the rate of about 4 per min. The minor fluctuations shown on this tracing are those of the respiratory rhythm. On two occasions the cannula became blocked and was cleared by flushing, after which the pulsations began again. The lower tracing is from a control cannula inserted into subcutaneous fat adjacent to the cistern. It records only cardiac and respiratory rhythms. It will be seen that the minimal pressure maintained within the cistern between pulsations is slightly above that of the pressure recorded by the control cannula. This suggests that the cistern maintains a small but constant tone as well as contracting intermittently.

Conclusion

While these operative findings all seem to confirm the hypothesis that the lymphangioma is a closed system, by doing so they raise a further problem. If, as it appears, the abnormal vessels and cisterns of the lymphangioma are a closed system, not connected to the general lymphatics and not receiving a retrograde supply of lymph from them, whence comes the pressure within the lymphangiomatous vessels, responsible for their dilatation? The most likely source seemed to be the muscle coats of the cisterns.

Observation

(16) In two cases of typical L.C. a cannula was inserted into one of the cisterns at operation. Tracings were made of the pressure within the cisterns and this was shown to rise and fall with a steady but very slow pulse rate of about 4 to 8 beats per minute, entirely independent of that of the cardiovascular system and of respiration. This rate of pulsation is of the same order as that which has been observed (Kinmonth, 1972) in normal large lymphatics such as the thoracic duct (Figs 13 and 14).

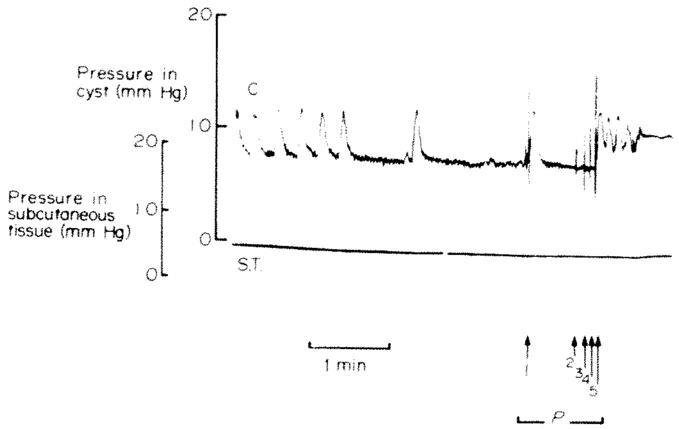


FIGURE 14. Similar tracing to show the response of a cistern to percussion. At the point P 1 the tissue adjacent to the cistern wall was gently tapped. The direct effect of this is shown on the tracing by a steep and instantaneous rise and fall in pressure, to which the cistern responded a few seconds later by a spontaneous contraction. At points P 2–5 a series of gentle taps was given, each progressively closer to the cistern wall. These are recorded as a series of increasingly steep instantaneous pressure rises. The response of the cistern to this stimulation was a burst of contractions at a higher than normal rate, and there was also a rise in the level of the minimal pressure maintained in the cistern, i.e. an increase in tone.

Conclusion

All these observations made *in vivo* support the morbid anatomical hypothesis that the abnormal vessels of L.C. stem from one or more underlying cisterns, and extend it by showing that their muscle coats pulsate slowly, causing fluctuation of the pressure within them. It seems likely that this pressure is transmitted from the cisterns, up through the abnormal subcutaneous and dermal lymphatics to reach the surface where the vesicles occur.

NATURAL HISTORY

The commonest history given in cases of L.C. is that a few vesicles were noticed in the skin at or shortly after birth, and that these have increased in number and in the area of skin they occupy during subsequent years. Other cases have a history that at birth there were neither vesicles nor any other abnormality detected and that the first sign of the lesion was the development of vesicles several years after birth. There is, however, a third group of cases which is perhaps the most enlightening. In these, of which ten have been encountered in the present series, the child at birth was observed to have a deep subcutaneous cystic swelling, usually situated either in relationship to the axilla or the base of the neck, or in the vicinity of the groin or buttock. At birth this swelling was the only abnormality detectable and there were no vesicles present in the overlying skin (Fig. 15a). These deep congenital cysts have on occasions been drained by aspiration or incision and in these cases they have fairly rapidly refilled. In more than one instance the axilla was explored surgically shortly after birth and the largest of the cysts removed intact, but it was noted at operation that others were present deeper in the axilla and these were left *in situ*. The cysts which were excised showed histologically that they had the typical appearances of lymphangiomatous cisterns, with a thick muscular wall (Fig. 15b). These deep cystic swellings which are present at birth seem to correspond to the condition generally known as cystic hygroma. The point of interest about these cases is that when followed for a

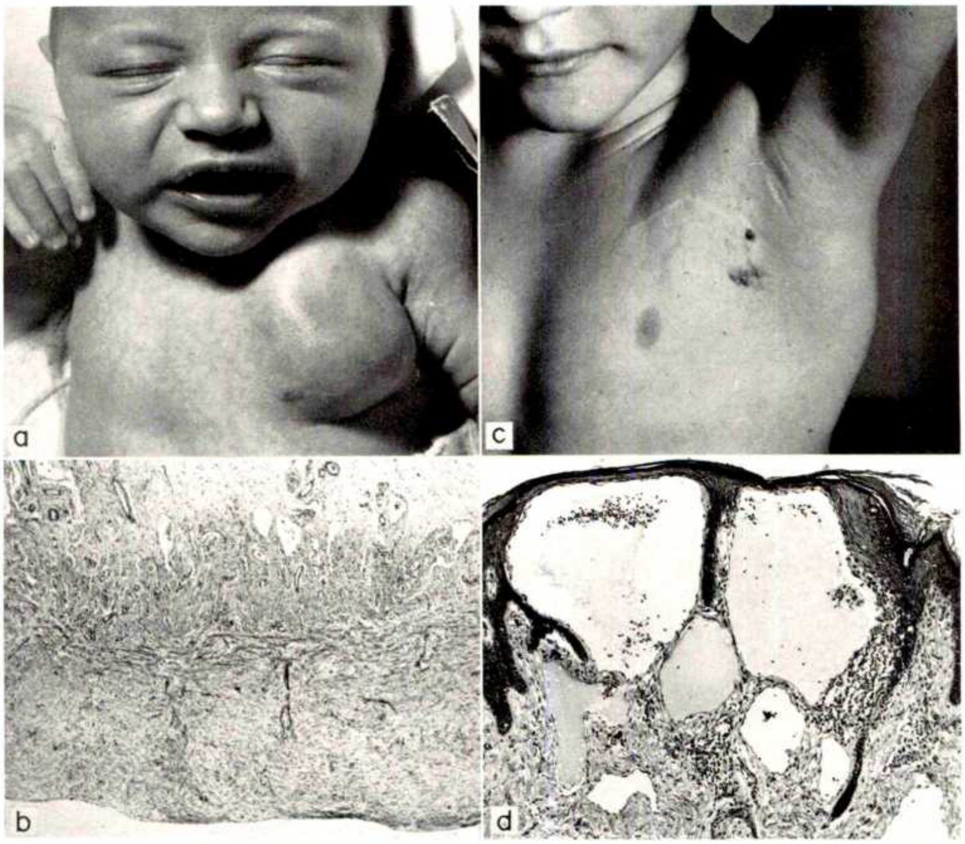


FIGURE 15. (a) Neonatal infant with congenital cysts deep in the subcutaneous tissue of the left upper chest wall. This region was explored surgically shortly after birth and the largest cyst removed. But others, smaller and deeper in the axilla, were left *in situ*.

(b) Section through the wall of the cyst removed from the infant shown in (a). In the collapsed and fixed state its wall was from 1 to 2 mm thick and its diameter 20 mm. The wall is formed of fibrous tissue liberally interlaced with smooth muscle. Small vessels can be seen traversing the wall, issuing outside it as dilated thin-walled lymphatics. The structure of this cystic hygroma is indistinguishable from that of an L.C. cistern.

(c) The same child as in (a) but 8 years later. Typical L.C. vesicles have developed near the scar.

(d) Histology of vesicles from the child as shown in (c). They have developed in skin which at birth appeared normal.

few years typical L.C. vesicles have developed in the skin overlying the congenital cysts, or the site from which the cysts had been partially excised (Fig. 15c, d). From these observations of the natural history it seems possible that in all cases the deep cisterns of the lesion are present at birth and that in some cases they have already given rise to overlying vesicles by that time. But in other cases, while the cisterns are detected at birth as deep swellings, the vesicles do not become apparent for a year or more afterwards. It may well be supposed therefore that there are other cases, perhaps the majority, in which subcutaneous cisterns are present at birth but not large enough or in such a position as to call attention to themselves. In such cases the first indication of the abnormality would be the development of vesicles later.

PATHOGENESIS

As has been stated above there are certain cases in which the natural history strongly suggests that the primary and congenital component of the L.C. lesion is the deep one. It can therefore be put forward as a hypothesis that the child is born with one or more isolated cisterns in the deep subcutaneous plane, and with an otherwise normal lymphatic system. It is a matter for conjecture as to how the cisterns might arise, but perhaps the most likely possibility is that they represent embryonic lymph sacs which have failed to link up with their fellows. These sacs develop initially in the embryo as separate structures and become interconnected later to form a continuous system of vessels.

As has been pointed out before (Peachey *et al.*, 1970) the majority of L.C. lesions are situated in relationship to the base of a limb and/or that part of the trunk to which it is connected. That is to say they occupy the regions from which limb buds developed in the embryo. It is thought possible that the sequestration of one or more lymph sacs in the embryo may occur at an early stage of limb development, and that once this has occurred the foundation of a future lymphangioma has been laid.

Pursuing this hypothesis it could be supposed that a wholly new and abnormal set of superfluous vascular channels, with dilated lumina and hypertrophied walls, grow out from the congenital cistern towards the skin, as a kind of 'blow-out' phenomenon secondary to the pressure created by the pulsation and tone of the muscle coat of the cistern. Such vessels would have no real circulation in them, only at best an ebb and flow, and they could not function as a drainage system for the skin.

Therefore to make this hypothesis compatible with the absence of oedema in lymphangiomatous skin it would have to be supposed that in addition to the abnormal, dilated, functionless vessels arising from the cisterns the skin also contains a system of normal lymphatics with proper central connections responsible for its drainage.

Observation

(17) Dye was injected intradermally into macroscopically normal skin within an area of L.C. When the subcutaneous tissues were subsequently explored in the quest for cisterns it was observed by the surgeon that there was a normal number of normal sized lymphatics draining dye from the injection site. These ran down through the subcutaneous fat alongside the dilated abnormal lymphatics of the L.C. There was no connection between the normal and the abnormal vessels and neither the abnormal vessels nor the cisterns contained dye.

Conclusion

Within an area of L.C. there are two intermingled but separate sets of lymphatics, one normal in structure and in its function of draining the skin; the other dilated, hypertrophic and seeming to perform no useful function as a drainage system.

THERAPEUTIC IMPLICATIONS

If it is accepted that the primary fault underlying L.C. is the congenital presence of isolated muscular cisterns, and that the abnormal vessels leading from them towards the skin surface are blind outgrowths from the cisterns, formed in response to their tone and pulsation pressure, then this will have two immediate implications concerning therapy.

(1) It must be regarded as futile to remove the skin containing the vesicles and a partial thickness of subcutaneous fat containing abnormal and dilated vessels (i.e. the tissue above the arrows in Fig. 10a, b) if the cisterns 'feeding' them are left *in situ*. Following such incomplete excision the original cisterns will simply sprout new abnormal vessels and subsequently give rise to new vesicles, as in Figs 3 and 4.

(2) If the cisterns are indeed the primary cause of all the more superficial abnormalities it may well prove to be adequate to remove just the cisterns (i.e. the tissue below the arrows in Fig. 10a, b) and leave the skin and upper subcutaneous layer intact. If the cisterns are the driving force behind the development of the other parts of the lesion (as the natural history of certain cases suggests) their removal might allow these other parts to involute. The cosmetic results of this type of selective deep excision, with skin preservation, would clearly be better than those of total radical excision. Excision of all the affected skin and subcutaneous tissue down to the deep fascia would in many cases (e.g. Fig. 1) be a mutilating procedure. The high recurrence rate after surgery in the past may to a large extent be attributed to surgeons erring on the conservative side, but misguidedly tending to conserve the most important part of the lesion, the deep part.

In nine cases to date surgery has been aimed at removing as many as possible of the cisterns and preserving as much as possible of the overlying skin. Quite small ellipses of skin containing half or much less of all the vesicles present have been excised, the margins of the wounds undercut in all directions and the underlying cisterns located and removed down to the level of the deep fascia. The wounds have then been closed, leaving vesicles still present in the adjacent skin. The results have so far been the exact opposite to those shown in Figs 3 and 4. No new vesicles have formed and those that remained in the skin after operation have disappeared. An account of the longer follow-up of patients treated by this method will be made the subject of a future publication.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to the various dermatological and surgical colleagues who in one way or another have steered excision specimens of L.C. into his hands; especially to Professors N.L.Browse and J.B.Kinmonth, without whose assistance this study could not have progressed beyond the armchair stage. Particular thanks are due to Professor Browse for carrying out, at the author's request, the procedures on which observations 16 and 17 were dependent.

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REFERENCES

KINMONTH, J.B. (1972) *The lymphatics*. Edward Arnold, London.

PEACHEY, R.D.G., LIM, C-C. & WHIMSTER, I.W. (1970) Lymphangioma of skin. A review of 65 cases. *British Journal of Dermatology*, **83**, 519.

British Journal of Dermatology (1976) **94**, 487.

Vascular changes in human skin after ultraviolet irradiation

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SUMMARY

Blood-flow changes in human skin after ultraviolet irradiation at 250 and 300 nm have been measured by three separate methods. Those methods which measure overall blood-flow changes in the skin showed increased flow after irradiation at both wavelengths. A method which measured flow only in the superficial vessels showed a slight increase in flow after low doses of both wavelengths, but in contrast, after higher doses, this method showed a marked reduction in flow through the upper dermal vessels. This reduction in flow is probably due to stasis in these superficial vessels, perhaps secondary to vascular damage. Contrary to previous reports, blood-flow changes after irradiation at 250 and 300 nm are similar and may be mediated by identical mechanisms.

Although the mechanism responsible for the development of erythema following ultraviolet irradiation (UVR) is unknown, two main theories have been postulated. The first is that UVR has a direct dilating effect on dermal blood vessels (Finsen, 1899), the second that UVR acts by releasing a vasoactive material which diffuses to the dermal blood vessels producing dilatation (Lewis, 1927).

It has been observed for many years that there are differences between the appearance of the erythema produced by different parts of the UV spectrum, and in the past the areas which have usually been chosen for study and comparison have been in the region of 250 and 300 nm, although 250 nm does not appear in terrestrial sunlight.

The studies of van der Leun (1966) have provided a great deal of information on vascular changes in the skin after 250 and 300 nm UVR. The results of his studies led van der Leun to conclude that 300 nm erythema was associated with arteriolar dilatation and increased blood flow in the skin, whilst 250 nm erythema was not. Furthermore, he suggested that erythema from these two different parts of the UV spectrum is produced by two different mechanisms.

Some earlier investigations (Ramsay & Cripps, 1970) indicated that vascular changes in the skin following UVR at 250 and 300 nm were identical, although these conclusions have been criticized (van der Leun, 1972).

We wish to report further studies on erythema induced by UVR at 250 and 300 nm using various methods of assessing changes in cutaneous blood flow.

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MATERIALS AND METHODS

Irradiation sources

We would have preferred to have used a monochromator for irradiating the skin at both wavelengths but the irradiated area obtainable from the prism monochromator available (Magnus *et al.*, 1959) is small and would have made vascular studies difficult. Repeated exposures would have been necessary to increase the size of the irradiated area leading to possible errors because of overlapping zones.

Radiation predominantly at about 250 nm was therefore provided by a low pressure or 'germicidal' mercury lamp (Philips TUV 30W) and at around 300 nm by a Westinghouse 20W fluorescent 'Sunlamp'. The lamps were aged for 100 h before use and the spectral emission was measured on a Beckman DBG recording spectrophotometer. These measurements showed that more than 98% of the emission from the 'germicidal' lamp was at the 253·7 nm 'line' whilst the 'sunlamp' had an emission between 285 and 360 nm with a peak at approximately 310 nm. Absolute intensity of the lamps was measured by chemical actinometry (Hatchard & Parker, 1956).

Subjects

The normal skin of the back of the upper trunk was used; sixteen subjects took part (10 males aged 33–70 years and 6 females aged 26–56 years). There was no history of photosensitivity in any of the subjects, who were either normal volunteers or patients suffering from localized dermatoses, e.g. hypostatic leg ulcers, well away from the irradiated area.

The minimal erythema dose (MED) for each source was determined for each subject by irradiating separate areas of skin, each 2 cm in diameter, with increasing doses. Results were read at 24 h and the lowest dose in the series which just produced erythema was taken as the MED.

Assessment of vascular changes

With each method, readings from the irradiated site were always compared with readings from an adjacent unirradiated site, each subject thus acted as his own control.

Skin thermometry

Thermistors were used, temperature being recorded directly with a Light Laboratories electric thermometer on which temperature changes of 0·05°C can be easily measured. The measured ΔT is the difference between skin temperatures at irradiated and adjacent unirradiated sites in each subject.

Photoelectric plethysmography

The method has been described in detail elsewhere (Challoner & Ramsay, 1974). ΔF is the difference in pulse height at irradiated and unirradiated sites in each subject.

Thermal conductance

The instrument used was that described by Challoner (1975). ΔC is the difference in thermal conductance at irradiated and unirradiated sites.

Experimental procedure

Eight subjects were examined with the 250 nm source and eight with the 300 nm source. With each source, reactions following both 2 and 5 times the MED were examined with the thermal conductance method but reactions from only 5 times the MED with skin thermometry and the photoelectric plethysmograph.

Each subject was examined before and where possible at 3, 6, 24, 48, 72 and 144 h after irradiation. In addition, with skin thermometry and photoelectric plethysmography, some subjects were examined 96 h after irradiation.

Each subject acted as his own control, readings from the irradiated site were always compared with readings from an adjacent unirradiated site. Thus it would be expected that if neither site was irradiated the result would be zero for skin thermometry and unity for photoelectric plethysmography and thermal conductance. In order to test this and to obtain a statistical analysis of the results, eight subjects were examined at exactly the same intervals as the irradiated group. Measurements were taken from one site on the back of the trunk and compared with an adjacent site. Thus the procedure in this group was exactly the same as in the irradiated group, except that the skin was not irradiated.

RESULTS

The MED with the 250 nm source varied between 37 and 74 mJ/cm^2 and with the 300 nm source between 160 and 320 mJ/cm^2. In the unirradiated group of eight subjects it is seen that the results vary around zero or unity (Figs 1–5). The results of the vascular studies were analysed statistically using the Wilcoxon Rank Test to compare irradiated and unirradiated groups.

250 nm source

Skin thermometry at 5 MED. Δ T increased 3 h after irradiation (Fig. 1), reached a maximum at 24 h and then slowly fell. The difference between the irradiated and control groups was statistically significant at all the time intervals with $P \leqslant 0{\cdot}05$.

Photoelectric plethysmography at 5 MED. Pulse height increased 6 h after irradiation (Fig. 2) and ΔF reached a maximum between 24 and 48 h ($P < 0{\cdot}05$ at all time intervals except at 3 h when the difference between the two groups was not significant).

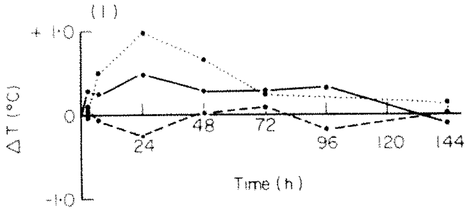
Blood volume also showed a rise reaching a maximum between 24 and 48 h (Fig. 3). Blood volume changes vary widely even in unirradiated skin and there was a wide scatter of observations in irradiated and unirradiated groups. We could obtain no statistically significant differences between the two groups.

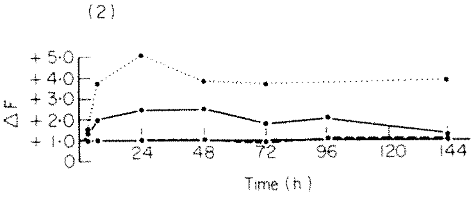
Thermal conductance at 2 and 5 MED. With a dose of 2 times the MED (Fig. 4), there was a rise with a maximum between 24 and 48 h. The difference at 48 h between irradiated and control groups was significant ($P < 0{\cdot}05$). However, with a dose of 5 times the MED (Fig. 5), a small rise at 6 h was followed by a fall below the control level by 24 h and this fall persisted throughout the period of observation. At 72 and 144 h, the irradiated group showed a significantly lower thermal conductance that the control population ($P < 0{\cdot}01$).

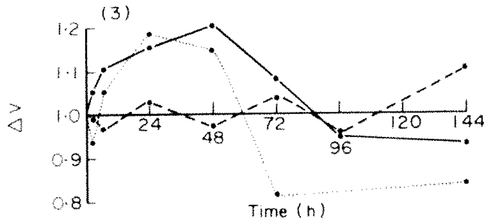
300 nm source

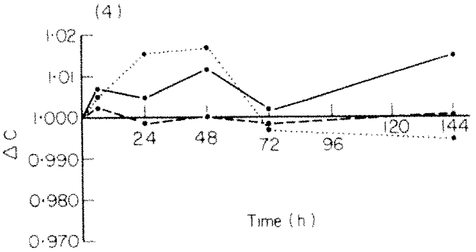
Skin thermometry at 5 MED. A rise in ΔT occurred by 6 h (Fig. 1) and reached a maximum by 24 h after irradiation. The rise was significant at all time intervals ($P < 0{\cdot}05$), except at 72 h.

Photoelectric plethysmography at 5 MED. Pulse height rose by 6 h (Fig. 2) and reached a maximum by 24 h. The rise in ΔF persisted throughout the period of observation and was significant ($P < 0{\cdot}05$) at all time intervals to 72 h.









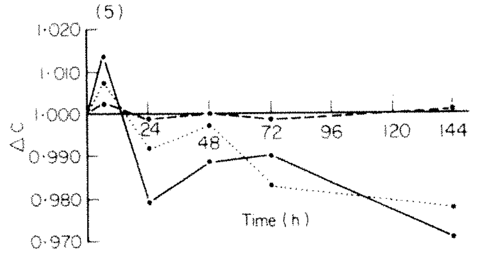


FIGURE 1. Skin temperature changes following irradiation with a dose of 5 × MED. The median of the series of results is plotted at each time interval. ——— Subjects irradiated with 250 nm UVR; .... subjects irradiated with 300 nm UVR; - - - - - unirradiated subjects.

FIGURE 2. Changes in pulse height in the skin (photo-electric plethysmograph) following irradiation with a dose of 5 × MED. Details and symbols as in Fig. 1.

FIGURE 3. Changes in blood volume in the skin (photo-electric plethysmography) following irradiation with a dose of 5 × MED. Details and symbols as in Fig. 1.

FIGURE 4. Thermal conductance changes in the skin following irradiation with a dose of 2 × MED. Details and symbols as in Fig. 1.

FIGURE 5. Thermal conductance changes in the skin following irradiation with a dose of 5 × MED. Details and symbols as in Fig. 1.

Blood volume (Fig. 3) also showed an increase maximal at 24 h ($P<0{\cdot}01$).

Thermal conductance at 2 and 5 MED. The results were rather similar to those with the 250 nm source. With a dose of 2 times the MED (Fig. 4) a rise occurred, maximum at 24–48 h ($P<0{\cdot}05$ at 24 h). With a dose of 5 times the MED (Fig. 5) a slight rise at 6 h was followed by a fall below the control level by 24 h, which persisted to 144 h.

DISCUSSION

There is no doubt that UVR produces dilatation of cutaneous blood vessels and Finsen (1899) suggested that the radiation had a direct action on the vessels. A second theory, that UVR acts by releasing a vasoactive material which then diffuses to the dermal vessels, was suggested by Lewis

(1927). For many years, the second theory has held sway (Blum, 1941; Partington, 1954) although there has been no direct experimental proof. Mackie & McGovern (1958) preferred the direct action theory of UVR on the dermis because they considered that the second theory failed to explain why the presence of melanin in the basal layer increased the erythema dose.

It has been observed for many years that there are differences between the appearance of the erythema produced by different parts of the UV spectrum. The two regions which have usually been chosen for study and comparison are at 250 and 300 nm. Terrestrial sunlight does not contain UVR below approximately 290 nm and so it is the erythema from 300 nm which most closely resembles natural 'sunburn'. Although 250 nm radiation is not present in terrestrial sunlight, the erythema which results from its action on the skin has some interesting differences from that produced by 300 nm. Erythema from 250 nm UVR is usually a pink-red, whilst that from 300 nm is a deeper red. Increase in dose of 250 nm leads to some increase in depth of colour of the erythema but this is much less marked than the increase in depth of colour following similar increase in dose of 300 nm (van der Leun, 1972). A definite bluish tinge to the erythema develops with increasing doses of 300 nm and this has been interpreted as showing involvement of deeper venules (Breit & Kligman, 1969). However, the results of experiments reported here suggest that a different mechanism may be responsible.

Although a great deal has been published on various aspects of UVR erythema (e.g. see Johnson, Daniels & Magnus, 1968) there has been little work on vascular changes from UVR of different wavelengths. One exception has been the studies of van der Leun (1966). Some earlier work suggested that blood flow in irradiated skin was increased (Lewis & Zotterman, 1926) but the dose employed was greatly in excess of the MED.

More recently, Holti (1955) showed a rise in skin temperature after UVR but again the dose used was high. Crockford, Hellon & Heyman (1962) used a strain-gauge plethysmograph to measure blood-flow changes in the skin of the forearm after UVR. No increase in flow was detectable before erythema developed and an increase in flow was not apparent until erythema had been established for 2–3 h. The strain gauge plethysmograph measures changes in volume in a segment of limb (i.e. skin, fat and muscle) and it is possible that small changes in skin blood flow might not be detected. Chimoskey, Flanagan & Holloway (1974) have recently demonstrated a rise in skin temperature and skin blood flow, as measured by ^{133}Xenon clearance, in man, after irradiation with a broad-band UVR source. These results using broad-band UVR therefore indicate that irradiation with UV in the region of 300 nm leads to vasodilatation and increased blood flow, but generally only after erythema is well established. However, the methods used for detecting changes in skin blood flow may not have been sufficiently sensitive to show the small changes which occur and these investigators have used different doses of UVR making it difficult to compare results from the different studies.

The work of van der Leun (1966) has indicated that two different parts of the UV spectrum may produce erythema by different mechanisms. The erythema from 300 nm radiation was associated with an increase in skin temperature, indicating an increase in cutaneous blood flow. On the other hand, van der Leun *never* found an increase in skin temperature with erythema from 250 nm radiation, even with doses as high as 30 times the MED. The results of the present study with 250 nm radiation are, therefore, completely at variance with the latter results of van der Leun. A dose of 5 times the MED of 250 nm radiation produced a rise in skin temperature which appeared 3 h after irradiation and which persisted for several days. We have also noted these temperature changes with doses as low as two times the MED. At the same time, the photo-electric plethysmograph showed a rise in pulse height which very closely followed the changes in skin temperature. We have, therefore, interpreted these results as indicating an increase in blood flow after irradiation with 250 nm UVR. The changes in skin blood flow, as indicated by temperature and pulse height measurements, following irradiation with 300 nm UVR, were very similar to those after 250 nm. A rise in both skin temperature and pulse height occurred about 3–6 h after irradiation and the time course of the changes was almost the same as that seen after irradiation with 250 nm UVR.

In addition to the very marked differences in skin temperature after 250 and 300 nm irradiation, van der Leun (1966) found other differences in the erythema from these two parts of the spectrum. An extension of the erythema beyond the irradiated area was definitely seen with 300 nm UVR but there was only minimal extension with 250 nm UVR. These differences led van der Leun to propose the following hypothesis. On the one hand, he suggested that 300 nm UVR produced erythema predominantly by affecting the epidermis in which a photo-chemical reaction occurred, producing the release of vasodilator material. On the other hand, 250 nm UVR, he proposed, affected the dermal vessels directly, producing dilatation. The vessels involved were thought to be the venules and not arterioles because of the lack of a detectable rise in skin temperature. He interpreted this latter finding, the absence of a change in skin temperature, as showing that skin blood flow did not rise after 250 nm UV irradiation. This hypothesis of 250 nm UVR acting directly on dermal vessels would appear to be at variance with observed measurements of transmission of UVR by the skin. It has been shown (Everett *et al.*, 1966) that, although the major part of the incident energy at 300 nm is absorbed by the epidermis, a small amount does reach the upper part of the dermis, whereas at 250 nm virtually all of the incident energy is absorbed by the epidermis.

The study reported here, where a definite rise in skin temperature did occur after 250 nm UVR, makes van der Leun's experimental findings difficult to explain. In addition, the rise in pulse height which followed a similar time course reinforces our view that blood flow after 250 nm UVR *is* increased.

The changes in thermal conductance measurements are of considerable interest. These were similar with erythema from both wavelengths, but there was a marked difference between the results at the two doses with each wavelength. With 300 nm UVR at 2 times the MED the maximal increase in blood flow occurred at 24–48 h after irradiation and with 250 nm UVR the increase of blood flow was maximal at 48 h. However, at 5 times the MED with both wavelengths, a slight increase in flow occurred at 6 h after irradiation, following which there was a definite reduction in flow. The reduction became more marked after 48 h, and at 144 h, flow was still considerably lower than the pre-irradiation level.

The methods used here for assessing blood flow in the skin measure changes at different levels in the dermis. The thermal conductance technique measures changes in the most superficial part of the dermis (Challoner, 1973) whilst skin thermometry and photo-electric plethysmography measure overall changes in both superficial and deeper vessels (Ramsay, 1973).

The results from these three methods may be used to formulate a new hypothesis concerning UV erythema. Skin temperature and pulse height changes both show that there is an overall increase in skin blood flow after irradiation with 250 and 300 nm UVR.

The thermal conductance experiments show that the increase in blood flow is also present in the most superficial part of the dermis at a low dose (2 times MED). At a higher dose (5 times MED) blood flow in the superficial dermis falls markedly but it is emphasized that at the same time blood flow in the deeper dermis is *increased* as measured by skin thermometry and photo-electric plethysmography. It is therefore suggested that both parts of the UV spectrum have an early effect on those vessels which control blood flow in the skin, *viz* vessels on the arterial side of the capillary. It is not known whether this effect is on the artery, arteriole, or the pre-capillary sphincter. The reduction in flow in the superficial dermis from higher doses probably results from stasis in the superficial vessels, perhaps secondary to vascular damage. This reduction in superficial flow is too small to influence skin temperature and pulse-height measurements, both of which reflect the overall increase in flow.

There is, therefore, no need to postulate two different mechanisms for the production of erythema from different parts of the UV spectrum. The simplest interpretation of the present study is that erythema from both parts of the spectrum have a common cause. Whether this is a direct action on the vessels or is secondary to the release of a vasodilator material from the epidermis is not known.

ACKNOWLEDGMENTS

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REFERENCES

BLUM, H.F. (1941) *Photodynamic Action and Diseases Caused by Light.* Hafner Publishing Co., New York.

BREIT, R. & KLIGMAN, A.M. (1969) Measurement of erythemal and pigmentary responses to ultraviolet radiation of different spectral qualities. In: *The Biologic Effects of Ultraviolet Radiation* (Ed. by F. Urbach). Pergamon Press, Oxford.

CHALLONER, A.V.J. (1973) Isotopic, optical and thermal conductance studies of skin vascularity changes induced by X-rays and UV-light. *Ph.D. Thesis.* Polytechnic of Central London.

CHALLONER, A.V.J. (1975) Accurate measurement of skin blood flow by a thermal conductance method. *Medical and Biological Engineering*, **13**, 196.

CHALLONER, A.V.J. & RAMSAY, C.A. (1974) A photo-electric plethysmograph for the measurement of cutaneous blood flow. *Physics in Medicine and Biology*, **19**, 317.

CHIMOSKEY, J.E., FLANAGAN, W. & HOLLOWAY, G.A. (1974) Ultraviolet-induced cutaneous hyperaemia measured by Xenon 133 in man. *Journal of Investigative Dermatology*, **63**, 367.

CROCKFORD, G.W., HELLON, R.F. & HEYMAN, A. (1962) Local vasomotor response to rubefacients and ultraviolet radiation. *Journal of Physiology*, **161**, 21.

EVERETT M.A., YEARGERS, E., SAYRE, R.M., & OLSON, R.D. (1966) Penetration of epidermis by ultraviolet rays. *Photochemistry and Photobiology*, **5**, 533.

FINSEN, N.R. (1899) *Ueber die Bedeutung der chemischen Strahlen des Lichts für Medicin und Biologie.* Vogel, Leipzig.

HATCHARD, C.G. & PARKER, C.A. (1956) A new sensitive chemical actinometer. II. Potassium ferrioxalate as a standard chemical actinometer. *Proceedings of the Royal Society A*, **235**, 518.

HOLTI, G. (1955) Measurements of the vascular responses in skin at various time intervals after damage with histamine and ultraviolet radiation. *Clinical Science*, **14**, 143.

JOHNSON, B.E., DANIELS, F., & MAGNUS, I.A. (1968) Response of human skin to ultraviolet light. In: *Photophysiology IV* (Ed. by A. C. Giese). Academic Press, New York.

LEWIS, T. (1927) *The Blood Vessels of the Human Skin and their Responses.* Shaw and Sons, London.

LEWIS, T. & ZOTTERMAN, Y. (1926) Vascular reactions of the skin to injury. VI. Some effects of ultraviolet light. *Heart.* **13**, 203.

MACKIE, B.S. & MCGOVERN, V.J. (1958) The mechanism of solar carcinogenesis. *Archives of Dermatology*, **78**, 218.

MAGNUS, I.A., PORTER, A.D., MCCREE, K.J., MORELAND, J.D. & WRIGHT, W.D. (1959) A Monochromator. An apparatus for the investigation of the responses of the skin to ultraviolet, visible and near infra red radiation. *British Journal of Dermatology*, **71**, 261.

PARTINGTON, M.W. (1954) Indirect effect of ultraviolet light on skin blood vessels. *Nature*, **174**, 134.

RAMSAY, C.A. (1973) Studies on vascular changes in the skin after ultraviolet radiation at 250 nm and 300 nm. *M.D. Thesis.* University of London.

RAMSAY, C.A., CRIPPS, D.J. (1970) Cutaneous arteriolar dilatation elicited by ultraviolet radiation. *Journal of Investigative Dermatology*, **54**, 332.

VAN DER LEUN, J.C. (1966) Ultraviolet erythema. A study of diffusion processes in human skin. *Ph.D. Thesis.* Utrecht.

VAN DER LEUN, J.C. (1972) On the action spectrum of ultraviolet erythema. In: *Research Progress in Organic, Biological and Medicinal Chemistry* (Ed. by L. Santamaria), Vol. 3, Part II, pp. 711–736. North Holland Publishing Co., Amsterdam.

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Phototoxic blisters from high frusemide dosage

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SUMMARY

Blistering of exposed areas has appeared in patients suffering from chronic renal failure treated with high doses of frusemide. The blistering, which resembles that occurring in nalidixic acid phototoxicity and in porphyria cutanea tarda, is considered phototoxic in nature.

Ebringer, Adam & Parkin (1969) reported bullous haemorrhagic eruptions in a patient suffering from chronic bronchitis and congestive cardiac failure which they attributed to high doses of frusemide (120 mg daily) taken for several weeks. The lesions which were present on the dorsal surfaces of hands, fingers, shins and feet disappeared when the diuretic ethacrynic acid was substituted. The authors discussed the possible relationship with bullous eruptions from barbiturates and sulphonamides, but did not express any opinion over the mechanism of bulla formation in their patient except to suggest that it was 'toxic' rather than 'allergic'.

An editorial in the *Lancet* (1971) headed 'big doses of frusemide in renal failure', indicates that as much as 500 mg of frusemide may be given hourly in the treatment of acute renal failure and that in high dosage the drug is also useful in chronic renal failure. Frusemide in doses not exceeding 2 g daily has been used for 4–5 years in the Renal Unit of the Queen Elizabeth Hospital in South Australia and has been associated with bullous lesions. Four representative cases are described and discussed in this paper.

Case 1

Chronic renal failure occurred in a 45-year-old housewife in September 1970. Treatment with frusemide 160 mg daily was increased to 1000 mg daily in August 1971, and in October 1971 the patient developed a haemorrhagic bullous eruption on the exposed dorsal surfaces of both feet and shins with minimal surrounding erythema (Fig. 1). Renal dialysis was commenced during the same month and a kidney transplant was carried out during January 1972. The bullous eruption of the legs had disappeared by then and did not return in spite of high intermittent intravenous doses of frusemide, as well as continuous oral frusemide (40 mg daily) during November and December 1972. She continued to take frusemide 40 mg daily until January 1974 when she died of cerebral infarction after thrombosis of the right carotid artery, revealed at autopsy when no skin lesions were noted.

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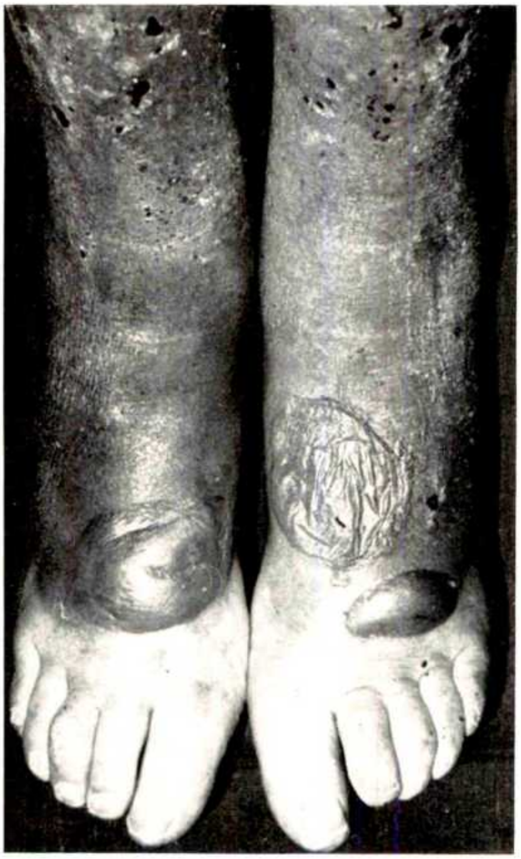

FIGURE 1. Blistering did not occur in those areas protected by slippers while she sat in the garden. Phototoxic blistering from nalidixic acid often occurs on the dorsal surfaces of shins and feet (Birkett *et al.*, 1969; Ramsay & Obreshkova, 1974).

Case 2

Chronic renal failure occurred in July 1970 in a 51-year-old housewife with polycystic kidney disease and diabetes mellitus. Frusemide 40 mg twice daily was taken until September 1971 when bendrofluozide 5 mg daily was substituted. This drug was stopped in November 1971 because of 'photosensitivity' diagnosed by her family doctor and replaced by 40 mg frusemide daily. In March 1972 the frusemide was increased to 120 mg daily and in spite of a further 'photosensitivity' reaction during May 1972, was increased during December 1972 to a daily dose of 250 mg. Frusemide 500 mg daily, started in January 1973, was increased to 1500 mg in August 1973 and blistering appeared on the backs of her hands and fingers soon afterwards. These persisted and one of them was biopsied in December 1973 (Fig. 2). Frusemide was replaced by ethacrynic acid and in spite of intermittent high dosage intravenous frusemide, there were no reports of blistering thereafter. A renal transplant was carried out during May 1974, but the patient died in July 1974.

Case 3

Chronic renal failure occurred in a 50-year-old draftsman in November 1971. After high dosage

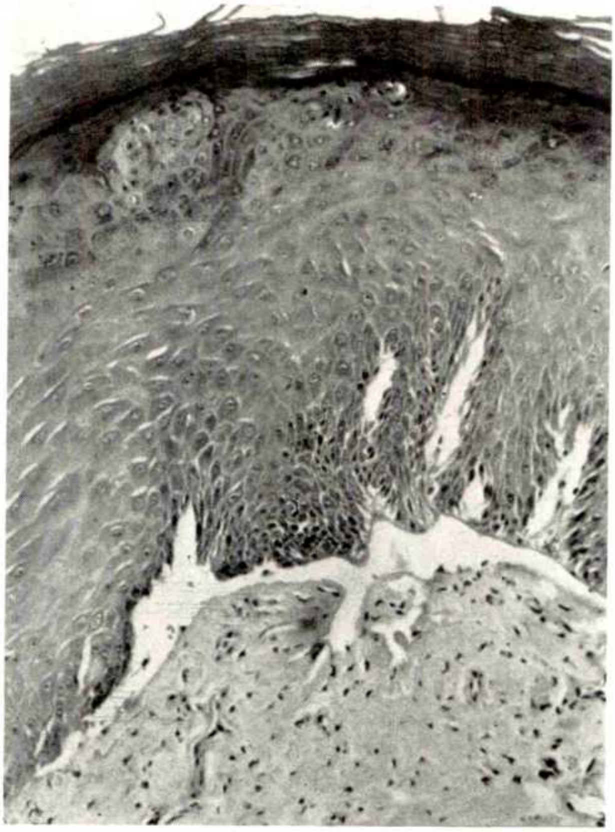

FIGURE 2. Part of the sub-epidermal blister biopsy which shows the over-all features. As in phototoxic blistering from nalidixic acid, there is very little inflammatory reaction (Birkett *et al.*, 1969; Ramsay & Obreshkova, 1974). As in porphyria cutanea tarda, both the rete ridges and the dermal papillae project into the cavity of the bulla (Milne, 1972).

intermittent intravenous frusemide outpatient therapy with 320 mg of the drug daily increasing to 480 mg was continued until April 1972 when dosage was increased to 750 mg daily. Blisters had been present on the backs of his hands (Fig. 3) since January 1972 and were associated with a general pruritus due to chronic uraemia. The blisters would heal without scarring, but recurred intermittently until May 1973, by which time the frusemide had been increased to 1 g twice daily. Investigations for porphyria were negative. The patient died in June 1973 and the cause of death was given after autopsy as chronic pyelonephritis associated with heart failure and generalized vascular disease.

Case 4

Chronic renal failure occurred in a 53-year-old housewife in 1967 due to chronic pyelonephritis associated with congenital spherocytosis and gout. Frusemide was given initially in small doses, but by August 1974 had reached 240 mg daily when blisters appeared on both hands (Fig. 4). Ethacrynic acid was substituted soon afterwards, but further blisters had appeared by late October 1974 when investigations for porphyria were negative. In spite of high dosage intravenous frusemide during hospitalization later in the year, the blisters did not recur and the patient's skin was clear during April and May 1975.

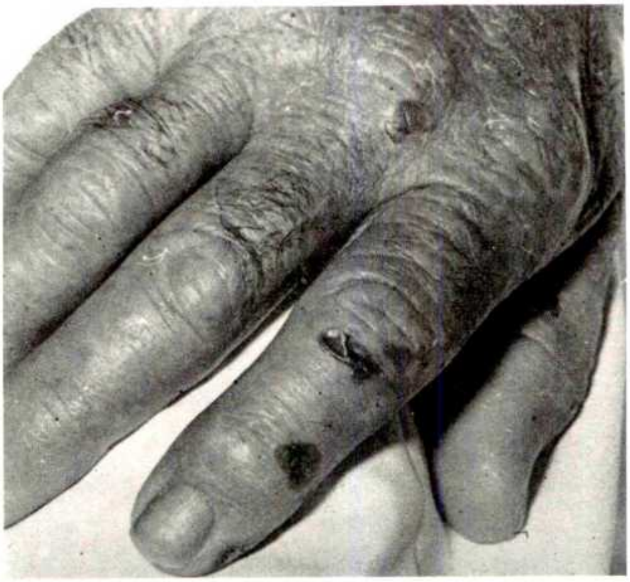

FIGURE 3. Healing blisters which very much resemble the appearances of porphyria cutanea tarda, whether caused by sunlight or trauma.

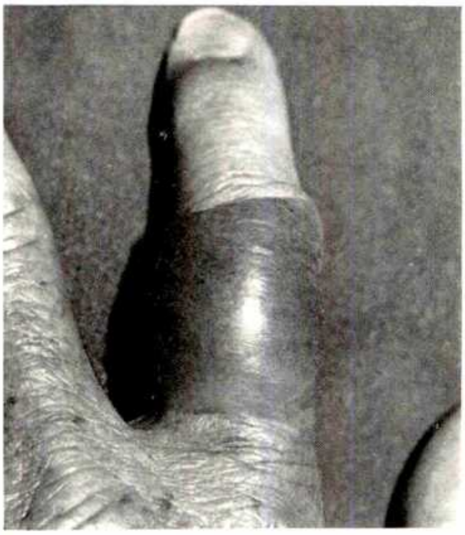

FIGURE 4. This single blister is of the acute stage when blisters are very often haemorrhagic.

DISCUSSION

The blisters on the dorsal surfaces of hands, fingers, shins and feet which occurred in our patients are similar, both in type of reaction and site of lesion, to phototoxic blisters from nalidixic acid (Birkett, Garretts & Stevenson, 1969; Ramsay & Obreshkova, 1974), although frusemide and nalidixic acid are quite different chemically. Ramsay & Obreshkova reported that in their patients a high level of ambient sunlight seemed to be more important than the length of time the nalidixic acid had been taken. In our cases the level of frusemide intake seemed to be of greater importance than the level of sunlight which is comparatively high in South Australia during all seasons. Like the blisters of nalidixic acid phototoxicity, the blisters in our patients were identical with those occurring in porphyria cutanea tarda, although there was no fragility of the uninvolved skin and the blisters healed without scarring. In two of our patients porphyria was excluded after investigations and in several other patients not included in this paper, it has been excluded similarly. The histology of the single biopsy was also identical with that occurring in porphyria cutanea tarda (Milne, 1972).

Because of repeated exposure to sunlight the backs of hands in Australians of European origin are susceptible to solar atrophy which decreases the protective skin layers to a greater degree than other exposed areas. We suggest this to explain the predominance of reactions on the backs of hands. In Case 4 the phototoxicity was persistent 1 month after frusemide had been stopped and resembled persistent phototoxicity from nalidixic acid (Birkett *et al.*, 1969; Burry, 1974; Ramsay & Obreshkova, 1974). All our patients suffered from chronic renal failure which may have contributed to a high level of frusemide or a frusemide metabolite in skin, a possible factor in the mechanism of bulla production. Similar reactions to both frusemide and chlorothiazide diuretics, both given in average dosage, which clinically suggest photoallergy, are seen occasionally in South Australia (Burry, unpublished observations).

REFERENCES

BIRKETT, D.A., GARRETTS, M. & STEVENSON, C.J. (1969) Phototoxic bullous eruptions due to nalidixic acid. *British Journal of Dermatology*, **81**, 342.

BURRY, J.N. (1974) Persistent photoxicity due to nalidixic acid. *Archives of Dermatology*, **109**, 263.

EBRINGER, A., ADAM, W.R. & PARKIN, J.D. (1969) Bullous haemorrhagic eruption associated with frusemide. *Medical Journal of Australia*, **i**, 763.

EDITORIAL (1971) Big doses of frusemide in renal failure. *Lancet*, **iv**, 803.

MILNE, J.A. (1972) *An Introduction to the Diagnostic Histopathology of the Skin.* Edward Arnold, London.

RAMSAY, C.A. & OBRESHKOVA, E. (1974) Photosensitivity from nalidixic acid. *British Journal of Dermatology*, **91**, 523.

British Journal of Dermatology (1976) **94**, 501.

Immunological aspects of psoriasis

1. IMMUNOGLOBULINS AND ANTI-IgG FACTORS

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SUMMARY

Sixty patients with psoriasis have been studied for quantitative estimation of serum immunoglobulin A, G and M (60 cases), immunoglobulin E (43 cases), salivary immunoglobulin A (28 cases) and anti-IgG factors in the serum (40 cases). All the data have been statistically compared to a large control group (300 subjects).

The results show an increase of the serum IgG and IgA means, an increase of salivary IgA which is correlated with the serum IgA, an increase of IgE levels in some patients and the presence of anti-IgG activity in the serum of 45% of patients with psoriasis. All these findings are statistically significant when compared to controls. These data could be in favour of auto-immune processes in psoriasis.

Many immunological studies have been performed in psoriasis. However, the results reported have been negative (Landau *et al.*, 1965) or conflicting. Serum immunoglobulin disturbances have already been described by many authors (Fraser, Dick & Grichton, 1969; Marghescu & Braun-Falco, 1970; Varelzidis & Theodoris, 1971; Schneider & Gurenci, 1972; Lai a Fat & Van Furth, 1974). Our group has previously published data concerning anti-IgG activity on blood lymphocytes of psoriatic patients (Rimbaud *et al.*, 1973).

This present work was undertaken to confirm the presence of such an activity in psoriatic serum. Likewise, we have carried out a complete study of serum and salivary immunoglobulins. All the results have been statistically compared with a large control group.

MATERIALS AND METHODS

Subjects. We have studied sixty patients with psoriasis (23 females and 37 males) with ages ranging from 16 to 90 years. According to the extent of psoriasis three groups of patients were separated: few lesions (group 1: nine patients); numerous disseminated lesions (group 2: twenty-nine patients);

erythrodermic or generalized psoriasis (group 3: twenty-two patients). An arthropathy was present in twelve patients, characterized by an inflammatory arthritis with bone destruction and reconstruction. All the patients were treated by topical therapy and no patient had received any cytotoxic drugs for a minimum of 6 months prior to this study.

Analysis for variance was used to compare the clinical features. Age did not correlate with extent of the psoriasis; those who had their disease for longer periods tended to be older than those who had it for shorter periods, and those with arthropathy tended to be older than those without.

Control groups. All the subjects in the control group were healthy blood donors. Comparisons were made also with forty-six patients with eczema.

Quantitative estimation of serum immunoglobulins (Ig). The quantitative determination of IgA, IgG and IgM in the serum was performed by the radial immunodiffusion technique (Mancini, Carbonara & Heremans, 1965), using commercially available plates (Beringwerke, Marburg/Lahn, Germany). Control serum was a reference serum no. 67/68 from World Health Organisation (WHO). Immunoglobulin E (IgE) was studied according to the method described by Rowe (1969) with a reference serum (batch no. 68-341 from WHO). All the results were expressed as International Units per ml (IU/ml). To permit comparisons with results from other authors, the immunoglobulin concentration was also calculated in mg/ml using the data of Rowe, Grab & Anderson (1972).

Salivary IgA determination (S-IgA). Specimens of saliva, stimulated with lemon juice, were collected using syringes and stored at −20 °C until processed. Quantitative estimation was carried out by radial immunodiffusion using commercially available plates (Hyland plates for low amounts). Results were expressed as mg/100 ml.

Anti-IgG factors in the serum. Anti-IgG factors were searched for in the serum of patients by an indirect immunofluorescence method previously described by Peltier *et al.* (1972). Briefly, human erythrocytes (group O rhesus negative) were sensitized by inactivated rabbit anti-human erythrocyte IgG. These IgG-coated erythrocytes were spread on a slide. Doubling dilutions of the patients' sera were put on the slides for 30 min at room temperature. Then the slides were stained by fluorescein conjugated sera: anti-human gammaglobulin serum (AHGS), anti-human IgA serum (AIgAS), anti-human IgG serum (AIgGS) and anti-human IgM serum (AIgMS). All these sera were obtained from Cappel Laboratories, Downingtown, Pa., and were checked for specificity by standard methods. A positive test was a serum dilution of more than 1/50.

Statistical tests. We have used six different statistical methods (Bahn, 1972): Student's '*t*'-test, Chi square test, Gauss–Laplace's test, One way analysis of variance studied by the ratio: group mean square/residual mean square, linear correlation according to Bravais–Pearson. Curvilinear correlations were obtained by transformation of variables to make the phenomenon linear (Aivazian, 1970). Then Pearson correlation test was calculated by the least square method.

RESULTS

Quantitative determination of serum immunoglobulins and S-IgA. The results obtained in sixty patients with psoriasis are given in Table 1. About 50% of psoriatics had elevated serum IgA and salivary IgA.

TABLE 1. Results of quantitative estimation of serum immunoglobulins and salivary IgA (S-IgA) in psoriasis expressed in International Units per ml (IU/ml)

	No.	Mean	s.d.	Elevated levels	No.	%
S-IgA	60	245·4	±99·1	⩾267	26	43
IgG	60	146·3	±41·1	⩾184	13	21
IgM	60	166·5	±83·6			
IgE	43	454·4	±996	⩾450	9	21
S-IgA	28	19·2*	±27·5*	⩾10*	15	53

* Results given in mg per 100 ml.

Comparisons were studied with the control group (healthy blood donors) (Table 2). Patients with psoriasis had levels of IgA, S-IgA and IgG which were significantly higher than controls (Student's '*t*'-test).

TABLE 2. Quantitative estimation of serum immunoglobulins and salivary IgA (S-IgA) in controls and psoriasis. The results are expressed as IU/ml for serum immunoglobulins and as mg/100 ml for S-IgA. Student's '*t*'-test was employed

	IgA		IgG		IgM		S-IgA	
	Controls	Psoriasis	Controls	Psoriasis	Controls	Psoriasis	Controls	Psoriasis
No.	300	60	300	60	300	60	40	28
Mean	144·6	245·4	127	146·3	176·7	166·5	5·2	19·2
s.d.	±58·9	±99·1	±27	±41·1	±66·2	±83·6	±1·19	±5·2
P	<0·00001		<0·001		NS		<0·00001	

When we compared serum immunoglobulins in psoriasis and in eczema, a significant difference was found for serum IgA and IgM. The level of serum IgA was higher ($P<0{\cdot}01$) in psoriasis (245·4±99 IU/ml) than in eczema (168·4±61 IU/ml). Serum IgG was increased in psoriasis (146·3±41 IU/ml) as well as in eczema (145·2±32 IU/ml) when compared to the control group ($P<0{\cdot}001$). Lastly, serum IgM was lower in eczema (145·3±67 IU/ml) than in either the control group ($P<0{\cdot}01$) or the psoriasis patients ($P<0{\cdot}01$).

Quantitative determination of serum IgE. Serum IgE was investigated in 727 controls and forty-three patients with psoriasis. A level higher than 450 IU/ml has been considered as abnormal and has been found in fifty-seven controls and nine psoriasis patients. The values in the latter group ranged from 452 to 6000 IU/ml. Comparative analysis (by chi square test) of subjects with elevated levels showed a significant difference between the control (7·8%) and psoriatic (20·9%) groups.

Anti-IgG factors in the serum. Anti-IgG factors were investigated in sera from forty patients with psoriasis and fifty age-matched subjects without psoriasis or any rheumatic disease. All the controls were negative when studied with fluorescent AHGS, AIgAS, AIgGS and AIgMS. In eighteen of forty psoriatics (45%) a distinct immunofluorescence was noted with AHGS and AIgGS but never with AIgAS and AIgMS. Thus an anti-IgG activity in serum IgG was shown.

Correlations with clinical features. We have especially investigated any relationship between the serum IgA levels which were increased and the age of the patients, the extent of the lesions, the onset of the disease and any psoriatic arthropathy.

Three age groups of control subjects and of patients with psoriasis were made. It was noted that a significant difference existed between the mean level of serum IgA in less than 30-year-old control subjects (138·6 ± 57 IU/ml) and in controls over 60 years (160·7 ± 58 IU/ml). There was an increase of this immunoglobulin with age. A similar difference was demonstrated in the psoriasis group between patients under 30 years (156·9 ± 36 IU/ml) and over 60 years (278·1 ± 100 IU/ml). However, in spite of this, there was a highly significant difference between the control group and the psoriasis group in those aged 30–60 years (145 ± 56 IU/ml versus 225·4 ± 84 IU/ml) and over 60 years (160·7 ± 58 IU/ml versus 278·1 ± 100 IU/ml).

The comparison between serum IgA and the extent of the lesions, according to the criteria previously defined, showed that IgA was significantly higher in patients of group 3 (generalized psoriasis: 291·1 ± 121 IU/ml) than in group 1 (few lesions: 194·9 ± 72 IU/ml) and in group 2 (numerous disseminated lesions: 220·8 ± 70 IU/ml).

Likewise, serum IgA was more elevated in patients whose disease had persisted for more than 10 years than in patients with recent onset of psoriasis.

Lastly, there was a significant difference between serum IgA levels of patients with (315·8 ± 125 IU/ml) and without (223·7 ± 85 IU/ml) psoriatic arthropathy.

No positive correlation was found between the presence of anti-IgG factors in the serum and the age of the patients, extent of the eruption, the onset of the disease or the presence of arthropathy. Among the eight patients with arthritis three were positive for anti-IgG factors.

Correlations between biological data. Three statistical studies were performed. A significant exponential correlation was reported between serum IgA and S-IgA (28 cases, $r = 0{\cdot}407$, $P < 0{\cdot}05$). The increase of these two parameters progressed in the same way. Furthermore, there was an exponential positive correlation between serum IgA and serum IgE (43 cases, $r = 0{\cdot}437$, $P < 0{\cdot}01$). Lastly, neither linear or curvilinear relation was found between the levels of anti-IgG factors and of the serum IgG.

COMMENT

This work emphasizes some disturbances of the immunoglobulins in psoriasis:

An increase of serum IgG and IgA, the latter being correlated with some clinical data (extent of lesion, onset of the disease, presence of psoriatic arthropathy); an increase of salivary-IgA levels which are correlated with those of serum IgA; an increase of IgE titres in some patients with psoriasis, which are correlated with those of serum IgA; the presence of anti-IgG activity in the serum of 45% of patients with psoriasis.

All these data are statistically significant when compared with controls. This study was performed on sixty patients with psoriasis who were having no treatment during the time of blood sampling and on 300 controls.

Comparison with other studies. Many reports of quantitative estimation of serum immunoglobulins have been published (Table 3). The results are conflicting, but IgA has usually been increased in psoriasis. In the studies of Schneider & Gurenci (1972) and Lai a Fat & Van Furth (1974) these immuno-

TABLE 3. Comparisons of serum immunoglobulin levels in psoriasis as found in the present study and reported in the literature (↗, raised; ↘, lowered; =, normal)

	Fraser (1969)	Marghescu (1970)	Varelzidis (1972)	Schneider (1972)	Lai a Fat (1974)	Present study	
No.	27	101	105	20	15	60	
IgA	↗	↗	↗	↘	↘	↗	Probable increase
IgG	↘	↗	=	=	↘	↗	?
IgM	↘	=	=	=	↘	=	?

globulins were depressed, but the groups were small. On the other hand, no conclusion can be given for the serum IgG and IgM. Our findings concerning salivary-IgA agree with those of Oon *et al.* (1973) who reported values in twelve psoriatics double those in the control group.

Few investigations have been reported concerning serum IgE in psoriasis. Juhlin *et al.* (1969) in six cases, Gurewith, Heiner & Reisner (1973) in eleven cases and Ogawa *et al.* (1971) in twenty-one cases described normal mean values. However, the wide range of normal and pathological levels is such that we think that it is better to compare the percentage of high IgE levels in psoriasis to that in controls. We find 21% of pathological levels in psoriasis and 7·8% in controls (727 control subjects). The difference is statistically significant. This difference seems to be present in Ogawa *et al.*'s study if we look at their Table 3 (Ogawa *et al.*, 1971).

We have found an anti-IgG activity in the serum of 45% of psoriatic patients. Such an activity was not found in psoriatic arthropathy by Tapanes, Rawson & Hallander (1972), but has been recently described by Howell *et al.* (1972). Krogh & Tonder (1972) have found anti-IgG factors in psoriatic lesions and Rimbaud *et al.* (1973) have reported a similar anti-IgG activity on peripheral blood lymphocytes. The classical serological reactions for rheumatoid factor detect mainly agglutinating antibodies and are always negative in psoriatic arthropathy. The immunofluorescence method and the technique of absorption–elution developed by Torrigiani & Roitt (1967) permit the demonstration of IgG anti-IgG antibodies which are not agglutinating.

Significance of immunoglobulin disorders. The significance remains uncertain. The mean feature is the increase of serum and salivary IgA. Many dermatoses with an inflammatory component have elevated IgA levels, e.g. dermatitis herpetiformis, acne vulgaris, rosacea and discoid lupus erythematosus (Fraser *et al.*, 1969). This increase could be due to cutaneous inflammation, since it is correlated with the extent of the skin involvement. However, in our eczema group, in which the inflammatory extent was similar, the IgA levels were less increased. The difference between psoriasis and eczema remains significant for the IgA mean levels but not for the IgG mean levels. It is thus likely that the IgA increase is specific for psoriasis.

There are various explanations for this phenomenon. First, the IgA elevation could reflect a general hyperfunction of immune responses as can be seen in some auto-immune diseases. Many studies in animals have demonstrated that the serum IgA concentration is affected by thymectomy and that the IgA antibody response to certain antigens is closely thymus dependent (Tomasi & Grey, 1972). Alternatively, the thymus may have a more direct influence on the secretory antibody producing system. Perhaps an unknown link exists between IgA production and thymus or T-cell functions.

Another possibility is that the IgA increase reflects an antibody response to a hypothetical infectious agent. The elevation of both salivary and serum IgA could be due to the passage of serum IgA through the mucosa, but local synthesis following antigenic stimulation seems more likely (Tomasi & Grey, 1972). It is well known that IgA antibodies are particularly effective in virus neutralization.

Concerning IgE, it is of interest to note a close correlation between its increase and that of serum IgA. These two disturbances could have the same origin or could be dependent. A relationship between these immunoglobulins has recently been reported in atopic eczema (Taylor *et al.*, 1973). Or some correlation between cyclic AMP and IgE disorders could exist in psoriasis. Voorhes *et al.* (1972) have reported a decrease of cyclic AMP in the psoriatic skin. However, these findings have not been confirmed by others (Harkonen, Hopsu-Havu & Raij, 1974). Lastly, it has been demonstrated that cyclic AMP inhibits IgE-mediated histamine release in the skin (Yamamoto, Greaves & Fairley, 1973).

The significance of anti-IgG activity in some psoriatic sera is unknown. The presence of such IgG antibodies has been reported in many other diseases including those due to auto-immune mechanisms. This finding, the anti-IgG activity bound to blood lymphocytes (Rimbaud *et al.*, 1973), the close correlation between psoriasis and HL-A antigens (Rimbaud *et al.*, 1974) and the increase of serum and salivary IgA could be in favour of auto-immune processes in psoriasis.

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REFERENCES

Aivazian, S. (1970) *Etude statistique des dependances.* Mir Ed. Moscou.

Bahn, A.K. (1972) *Basic Medical Statistics.* Grune and Stratton, New York.

Fraser, N.G., Dick, H.M. & Grichton, W.B. (1969) Immunoglobulins in dermatitis herpetiformis and various other skin diseases. *British Journal of Dermatology*, **81**, 89.

Gurevitch, A.W., Heiner. P.C. & Reisner, R.M. (1973) IgE in atopic dermatitis and other common dermatoses. *Archives of Dermatology*, **107**, 712.

Harkonen, M., Hopsu-Havu, V.K. & Raij, K. (1974) Cyclic adenosine monophosphate, adenyl cyclase and cyclic nucleotide phosphodiesterase in psoriatic epidermis. *Acta dermato-venerologica*, **54**, 13.

Howell, F.A., Chamberlain, M.A., Perry, R.A., Torrigiani, G. & Roitt, I.M. (1972) IgG antiglobulin levels in patients with psoriatic arthropathy, ankylosing spondylitis and gout. *Annals of Rheumatic Diseases*, **31**, 129.

Juhlin, L., Johansson, S.G.O., Bennich, H., Hogman, C. & Thyresson, M. (1969) Immunoglobulin E in dermatoses. Levels in atopic dermatitis and urticaria. *Archives of Dermatology*, **100**, 12.

Krogh, H.K. & Tonder, O. (1972) Immunoglobulins and anti-immunoglobulin factors in psoriatic lesions. *Clinical and Experimental Immunology*, **10**, 623.

Lai a Fat, R.F.M. & Van Furth, R. (1974) Serum immunoglobulins levels in various skin diseases. *Clinical and Experimental Immunology*, **17**, 129.

Landau, J.W., Gross, B.G., Newcomer, V.D. & Wright, E.T. (1965) Immunologic response of patients with psoriasis. *Archives of Dermatology*, **91**, 607.

Mancini, G., Carbonara, A.O. & Heremans, J.F. (1965) Immunological quantification of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235.

Marghescu, S. & Braun-Falco, O. (1970) Quantitatives Verhalten von Immunoglobulinen und Komplement bei Psoriasis. *Archiv für Klinische und experimentelle Dermatologie*, **238**, 417.

Ogawa, M., Berger, P.A., McIntyre, O.R., Clendenning, W.E. & Ishizaka, K. (1971) IgE in atopic dermatitis. *Archives of Dermatology*, **103**, 575.

Oon, C.H., Goodwin, P.G., Kind, P. R. N., Seah, P.P. & Fry, L. (1973) Salivary IgA in patients with psoriasis and dermatitis herpetiformis. *Acta dermato-venereologica*, **53**, 340.

Peltier, A., Leroy, C., Atra, E. & Haim, T. (1972) Detection des facteurs antigammaglobuliniques (facteurs rhumatoïdes) seriques par immunofluorescence. *Pathologie et Biologie*, **20**, 181.

RIMBAUD, P., MEYNADIER, J., GUILHOU, J.J. & CLOT, J. (1973) Anti-IgG activity on peripheral blood lymphocytes in psoriasis. *Archives of Dermatology*, **108**, 371.

RIMBAUD, P., MEYNADIER, J., GUILHOU, J.J., CLOT, J. & SEIGNALET, J. (1974) Troubles immunitaires et antigènes d'histocompatibilité dans le psoriasis. *Annales de Dermatologie et Syphiligraphie*, **101**, 359.

ROWE, D.S. (1969) Radioactive single radial diffusion: a method for increasing the sensitivity of immunochemical quantification of protein in agar gel. *Bulletin of World Health Organisation*, **40**, 613.

ROWE, D.S., GRAB, B. & ANDERSON, S.G. (1972) An international reference preparation for human serum immunoglobulins G, A and M. *Bulletin of World Health Organisation*, **46**, 67.

SCHNEIDER, C. & GÜRENCI, J. (1972) Serumprotein bestimmungen mittels radialer Immunodiffusion bei verschsedenen Dermatosen. *Hautarzt*, **23**, 209.

TAPANES, F.J., RAWSON, A.J. & HOLLANDER, J.L. (1972) Serum anti-immunoglobulins in psoriatic arthritis as compared with rheumatoid arthritis. *Arthritis and Rheumatism*, **15**, 153.

TAYLOR, B., NORMAN, A.P., ORGEL, H.A., STOKES, C.R., TURNER, M.W. & SOOTHILL, J.F. (1973) Transient IgA deficiency and pathogenesis of infantile atopy. *Lancet*, **ii**, 111.

TOMASI, T.B. & GREY, H.M. (1972) Structure and function of immunoglobulin A. *Progress in Allergy*, **16**, 81.

TORRIGIANI, G. & ROITT, I.M. (1967) Antiglobulin factors in sera from patients with rheumatoid arthritis and normal subjects. *Annals of the Rheumatic Diseases*, **26**, 334.

VARELZIDIS, A. & THEODORIS, A. (1971) Serum immunoglobulins in psoriasis before and after ultraviolet light therapy. *British Journal of Dermatology*, **105**, 695.

VOORHEES, J.J., DUELL, E.A., BASS, L.J., POWELL, J.A. & HARREL, E.R. (1972) Decreased cyclic AMP in the epidermis of lesions of psoriasis. *Archives of Dermatology*, **105**, 695.

YAMAMOTO, S., GREAVES, M.W. & FAIRLEY, V.M. (1973) Cyclic AMP induced inhibition of IgE mediated hypersensitivity in human skin. *Immunology*, **24**, 77.

British Journal of Dermatology (1976) 94, 509.

Clinical response of dermatitis herpetiformis skin lesions to a gluten-free diet

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SUMMARY

Patients with dermatitis herpetiformis have been studied prospectively for 2 years to assess the effect of a gluten-free diet (GFD) on control of the skin lesions. Daily requirements for oral medication with sulphapyridine or dapsone were reduced by GFD treatment and if complete clinical remission of the skin disease occurred, it was maintained while the diet was strictly observed. However, complete remission did not occur significantly more often in GFD-treated patients than in patients taking a normal diet. Many of the latter group exhibited variation in their drug dose requirements during the period of study.

GFD treatment seems desirable for the majority of patients with dermatitis herpetiformis, not only to correct the intestinal abnormality but also to minimize the dose of drugs necessary to control the skin lesions.

Since the original demonstration of intestinal villous atrophy in patients with dermatitis herpetiformis (Marks, Shuster & Watson, 1966) there has been general agreement that the intestinal lesion responds to treatment with a gluten-free diet (GFD) in a manner comparable with adult coeliac disease (Fry *et al.*, 1968; Marks & Whittle, 1969; Weinstein *et al.*, 1971). However, there have been differences of opinion concerning the effect of GFD treatment on the skin which range from reports of little or no effect (Weinstein *et al.*, 1971; Shuster, Marks & Watson, 1968) to the finding of improvement in almost all patients (Fry *et al.*, 1973). It is obviously desirable that the consequences of GFD treatment in dermatitis herpetiformis should be clarified and we now report the findings of a 2-year prospective study of the effects of the diet on the skin.

PATIENTS AND METHODS

Forty-five patients with dermatitis herpetiformis were studied. All had initially been seen at general dermatological clinics in the South East Region of Scotland where a diagnosis of dermatitis herpetiformis had been made on the clinical and histological nature of the skin lesions and their response to treatment with either sulphapyridine or dapsone, according to the preference of the clinician concerned. The patients were then referred to us for further haematological, gastrointestinal and immunological investigation and for supervision of therapy. On their first attendance at our clinic the reasons for these further investigations were explained and the patients' co-operation in our studies invited. In addition, all patients were asked gradually to reduce the daily dose of whichever drug they were taking to the minimum required for control of their skin lesions.

After completion of the initial investigation and our acceptance of the diagnosis of dermatitis herpetiformis, the possible benefits of a GFD were explained. Ten patients were either unwilling to embark on the diet or because of their social circumstances would have found it particularly difficult to observe. These patients were therefore asked to continue with a normal diet. Nineteen patients agreed to begin a GFD immediately and a further sixteen patients agreed to do so after a control period of 6–12 months on a normal diet. The 2-year period of study began either with commencement of the GFD or, for the ten patients continuing on a normal diet throughout, at the time the decision regarding the diet was taken. The patients were then seen jointly by two of us (one dermatologist and one gastro-enterologist) at 3, 6, 12, 18 and 24 months. Patients taking a GFD were also seen by the dietician. At each attendance, the patient's daily requirement of sulphapyridine or dapsone was noted and he was reminded to attempt gradual reduction of the dose to the minimum acceptable to him.

TABLE 1. Patient groups studied

Treatment	No. of patients and sex	Mean age (years) and age range at entry to study	Mean duration of disease (years) and range at entry to study	Jejunal biopsy appearances* before entry to study (Crosby capsule biopsy)
Normal diet + sulphapyridine	6 M 4 F	49 (29–78)	4 (0–24)	1 SVA 4 PVA 2 normal 3 not biopsied
Normal diet + dapsone	3 M 3 F	52 (27–72)	12 (5–17)	2 SVA 2 PVA 2 normal
Gluten-free diet + sulphapyridine	7 M 3 F	52 (29–65)	5 (1–16)	4 SVA 4 PVA 1 normal 1 not biopsied
Gluten-free diet + dapsone	9 M 7 F	43 (14–66)	8 (1–15)	5 SVA 6 PVA 4 normal 1 not biopsied
Gluten-free diet 2 years + sulphapyridine or dapsone	3 M	47 (38–62)	10 (4–18)	2 SVA 1 PVA

* SVA = subtotal villous atrophy; PVA = partial villous atrophy (Shiner & Doniach, 1960).

Twenty-six patients observed the GFD strictly for the full 2-year period. Six who initially undertook to do so were either unwilling or unable to observe it strictly when seen at 3 months and were then instructed to resume a normal diet. These, together with the ten patients who were not asked to observe a GFD, constituted the 'normal diet' groups of the study.

Three patients did observe a GFD strictly for more than 3 months but defaulted before completing the 2-year period of the study. Details of the patient groups are summarized in Table 1.

RESULTS

The mean daily doses of sulphapyridine or dapsone required for control of the skin lesions are shown for each of the four main patient groups (Figs 1 and 2). The data are based on individual drug requirements noted at 243 clinic attendances by these forty-two patients; owing to non-attendance, individual requirements were not established on nine occasions.

Over the 2-year period of observation, the patients who were taking a normal diet apparently sustained a progressive fall in their mean daily drug requirement, although this fall does not attain statistical significance (paired '*t*' test on 0 and 24 month drug requirements, sulphapyridine group 't' $= 2{\cdot}05$, $P > 0{\cdot}05$; dapsone group 't' $= 1{\cdot}00$, $P > 0{\cdot}3$). Four of these patients had stopped all drug therapy by 24 months, without recurrence of their skin lesions and one has remained in remission for a further 2 years. Six patients reported no change in their drug requirement throughout the period of observation but the other six reported a varying requirement. One patient sustained a complete remission of his skin disease for 8 months during which he took no medication, but relapsed thereafter.

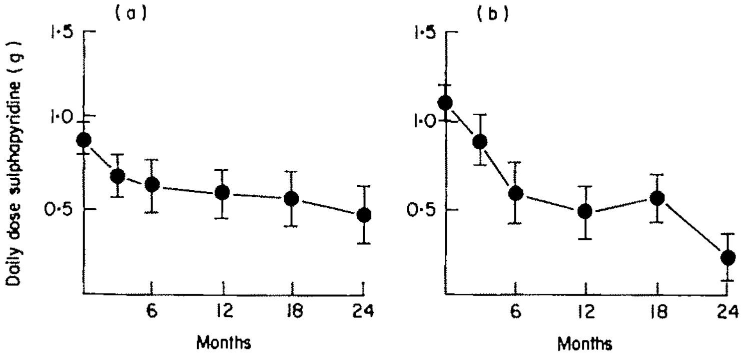

FIGURE 1. Daily sulphapyridine requirements for control of dermatitis herpetiformis skin lesions in (a) ten patients on a normal diet and (b) ten patients on a GFD. Data are means ± s.e.m.

The twenty-six patients who observed a GFD strictly for the full 2 years also exhibited a fall in their mean daily drug requirement. In these groups the fall attains statistical significance (paired '*t*' test on 0 and 24 month daily drug requirements, sulphapyridine group 't' $= 5{\cdot}31$, $P < 0{\cdot}005$, dapsone group 't' $= 3{\cdot}95$, $P < 0{\cdot}005$). Nine patients were able to stop medication completely, two within 6 months of beginning the GFD. After doing so, two patients relapsed briefly within the period of study and required further medication but all nine were in remission at 24 months and have continued to require no medication for periods ranging from a further 6 months to 2 years. Clinical remission of the skin disease was thus more frequent in GFD-treated patients (35%) than in the patients on a normal diet (25%) but the difference is not statistically significant.

Four patients taking a GFD reported no change in their daily drug requirement throughout the 2-year period. However, one of these patients was able to reduce his medication substantially after 30 months on the diet.

The data from the three patients whose observance of the GFD was initially strict but later erratic is not readily interpretable. All three reported a reduction in drug requirements some time after

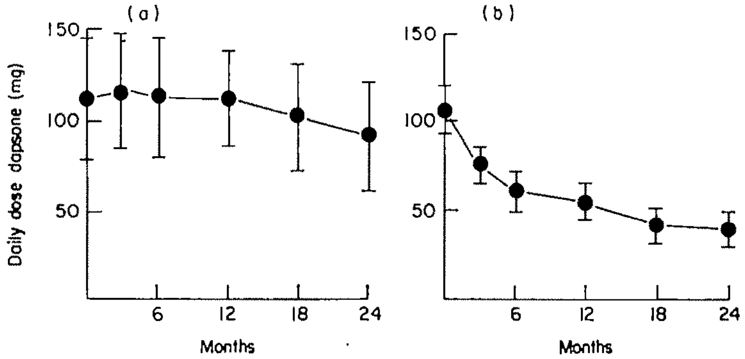

FIGURE 2. Daily dapsone requirements for control of dermatitis herpetiformis skin lesions in (a) six patients on a normal diet and (b) sixteen patients on a GFD. Data are means ± s.e.m.

beginning the diet and two reported an increase in requirement when their observation of the diet became lax. One patient then resumed a strict diet and again noted a fall in his drug requirement.

The demonstration of a statistically significant reduction in drug requirements over 24 months in the diet-treated group of patients is not necessarily evidence of a causal association between the diet and the reduction in drug dose. However, comparisons of the difference in daily drug requirements at 0 and 24 months exhibited by each patient indicate that a significantly greater reduction in drug requirements occurred in the patients taking a GFD (Mann Whitney U test, sulphapyridine groups $P<0{\cdot}01$, dapsone groups $P<0{\cdot}025$). Thus observance of the GFD was associated with a greater fall in drug requirement for control of the skin lesions and this suggests that the avoidance of dietary gluten was beneficial.

Strict observance of a GFD inevitably involves an element of self selection for such treatment by the patients themselves so that there is a possibility that the normal diet and GFD groups of patients are not strictly comparable. It is therefore of some importance to identify possible reasons other than the avoidance of dietary gluten which might account for the observed differences in drug requirements. We were particularly concerned by the possibility that the patients who maintained the GFD strictly might have been more conscientious in their attempts to reduce drug dosage and the monitoring of drug requirements prior to treatment with a GFD was therefore undertaken in an attempt to explore this possibility.

Drug requirements were monitored for a period of 6–12 months in sixteen patients before they began a gluten-free regime. Twelve subsequently observed the diet strictly for the full 2 years. Each of these patients was seen on at least three occasions before the GFD was introduced, and reminded of the previous instructions to reduce drug dosage to the minimum consistent with control of the skin lesions. The changes in their drug requirements during this period on a normal diet are illustrated (Fig. 3) and show no evidence of a falling requirement in the group as a whole.

DISCUSSION

The response of the skin lesions of dermatitis herpetiformis to treatment with a GFD has been the subject of some controversy. Although early reports described remission of the skin disease in some patients observing a GFD (Fry *et al.*, 1968; Van Tongeren *et al.*, 1967) the belief that, in general, a gluten-free regime allows healing of the intestinal lesion without affecting the skin has been widely held (Leading Article *BMJ*, 1970, 1971; Hardison, 1972). More recently, evidence that the skin is

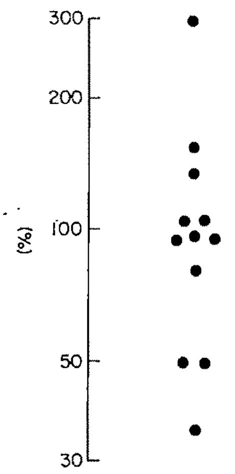

FIGURE 3. Daily sulphapyridine or dapsone requirements in twelve dermatitis herpetiformis patients after 6–12 months on a normal diet, expressed as % initial daily requirement. These patients subsequently observed a GFD strictly for 2 years.

almost always improved by GFD treatment has been presented (Fry *et al.*, 1973) but no confirmation of this has yet been reported.

The present results indicate that the observance of a strict gluten-free regime permits patients with dermatitis herpetiformis to maintain satisfactory control of their skin lesions with a lower average daily dose of sulphapyridine or dapsone than patients who continue with a normal diet. In this respect our findings accord with those of Fry *et al.* (1973). However, this observation is not in itself conclusive evidence that the diet is beneficial or that the skin lesions of dermatitis herpetiformis are gluten dependent. Inevitably patients must be motivated to strict observance of the gluten-free regime and we have little doubt that successful reduction in drug requirements provided our patients with reinforcement of that motivation. It is therefore possible that any patient whose drug requirements were falling spontaneously might gain encouragement to persist with the diet whereas those unable to reduce their medication have no such encouragement. If this were to lead to the latter group defaulting from strict observance of the diet, the resulting association between diet observance and falling drug requirements might be mistakenly interpreted as cause and effect.

This possibility is partially refuted by Fig. 3 which provides no evidence of a declining drug requirement during the control period in twelve patients who subsequently observed a strict GFD. Furthermore, failure to reduce drug dosage was not a cause of failure to observe the diet, since the three patients whose observance of the diet became lax all reported a reduction of their daily medication while adhering to the diet strictly. Thus it would seem that in our patients, the reduction in daily drug requirement was genuinely related to the avoidance of dietary gluten.

Our observations thus support the assertion (Fry *et al.*, 1973) that observance of a strict GFD minimizes the skin lesions of dermatitis herpetiformis and may lead to remission of the skin disease. We would also agree that in individual patients the benefits of the diet may take many months to become clinically apparent but our data nevertheless indicate that on average a progressive fall in daily drug requirements occurs from the time treatment is initiated.

We are unable to confirm the findings of Fry *et al.* (1973) with respect to the constancy of drug requirements in patients taking a normal diet. Our experience, like that of Marks & Shuster (1970), indicates that many patients taking a normal diet exhibit a variable requirement for their drugs and that some will go into clinical remission and require no medication. It is possible that these apparent differences in experience of the natural history of dermatitis herpetiformis arise in part from the application of different criteria for making the diagnosis. In the present study, the traditional criteria were used as the basis for diagnosis in all patients although these are inevitably both empirical and imprecise (Fry & Seah, 1974). Since our study was begun, the value of examining skin biopsies for IgA deposits has become increasingly apparent. Nevertheless, the precise diagnostic significance of such deposits is still not clear (Fry & Seah, 1974; Marks *et al.*, 1974).

Our data therefore indicate that, using traditional criteria for the diagnosis of dermatitis herpetiformis, spontaneous improvement and remission in the skin disease may occur, but may be associated with subsequent relapse. Improvement in the skin is much more likely in patients treated with a GFD, who are usually able to reduce their drug requirements and if complete clinical remission of the skin disease occurs, it is apparently maintained while the diet is strictly observed. Since a GFD may also minimize the risk of malignancy associated with gluten-sensitive enteropathy (Harris *et al.*, 1967), we feel it is appropriate therapy for the majority of patients with dermatitis herpetiformis.

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REFERENCES

FRY, L., MCMINN, R.M.H., COWAN, J.D. & HOFFBRAND, A.V. (1968) Effect of a gluten-free diet on dermatological, intestinal and haematological manifestations of dermatitis herpetiformis. *Lancet*, **i**, 557.

FRY, L., SEAH, P.P., RICHES, D.J. & HOFFBRAND, A.V. (1973) Clearance of skin lesions of dermatitis herpetiformis after gluten withdrawal. *Lancet*, **i**, 288.

FRY, L., SEAH, P.P. (1974) Dermatitis herpetiformis. An evaluation of diagnostic criteria. *British Journal of Dermatology*, **90**, 137.

HARDISON, W.G.H. (1972) In: *Yearbook of Dermatology* (Ed. by F.D.Malkinson and R.W.Pearson), p. 222, Chicago.

HARRIS, O.D., COOKE, W.T., THOMSON, H., WATERHOUSE, J.A.H. (1967) Malignancy in adult coeliac disease and idiopathic steatorrhoea. *American Journal of Medicine*, **42**, 899.

LEADING ARTICLE (1970) *British Medical Journal*, **iii**, 240.

LEADING ARTICLE (1971) *British Medical Journal*, **ii**, 295.

MARKS, J., SHUSTER, S. & WATSON, A.J. (1966) Small bowel changes in dermatitis herpetiformis. *Lancet*, **ii**, 1280.

MARKS, J. & SHUSTER, S. (1970) Dermatitis herpetiformis and gluten free diet. *British Medical Journal*, **i**, 498.

MARKS, J., SHUSTER, S., YOUNG, S., FELIX, R.H. & WATSON, A.J. (1974) The skin-gut relationship in dermatitis herpetiformis. *British Journal of Dermatology*, **91**, Suppl. **10**, 34.

MARKS, R. & WHITTLE, M.W. (1969) Results of treatment of dermatitis herpetiformis with a gluten-free diet after one year. *British Medical Journal*, **iv**, 772.

SHINER, M., & DONIACH, I. (1960) Histopathologic studies in steatorrhoea. *Gastroenterology*, **38**, 419.

SHUSTER, S., MARKS, J. & WATSON, A.J. (1968) Coeliac syndrome in dermatitis herpetiformis. *Lancet*, **i**, 1101.

VAN TONGEREN, J.H.M., VAN DER STAAK, W.J.B.M. & SCHILLINGS, P.H.M. (1967) Small bowel changes in dermatitis herpetiformis. *Lancet*, **i**, 218.

WEINSTEIN, W.M., BROW, J.R., PARKER, F. & RUBIN, C.E. (1970) The small intestinal mucosa in dermatitis herpetiformis. *Gastroenterology*, **60**, 362.

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Effect of the Hewitt keratinizing epidermal carcinoma on cell proliferation in different organs of the host mouse and in human psoriatic skin cultured in diffusion chambers

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SUMMARY

Incorporation of ^{3}H-thymidine into different squamous epithelia and other tissues was studied in mice bearing the Hewitt keratinizing epidermal carcinoma with a raised epidermal chalone content in the body. The results showed that DNA synthesis in the tumour-bearing mice was inhibited strongly in the epithelium of the ear (c. 80%), moderately in the epithelium of the back skin and the foot (c. 55%), and weakly in the epithelium of the tongue and the cornea (c. 25%). These inhibitions appear to be specific in nature, because cell proliferation was not detectably affected in the sebaceous gland, intestinal epithelium, bone marrow, and thymus lymphocytes. Thymus size, however, was reduced in the tumour-bearing mice compared with controls, and ^{3}H-thymidine uptake was depressed in the spleen and to some extent also in the lymph nodes, possibly owing to non-specific stress.

Labelling index of human psoriatic epidermis was suppressed in diffusion-chamber cultures carried by the tumour-bearing host mice compared with controls. However, this reaction was probably not specific in nature, because the labelling index of mouse bone-marrow cells was also decreased when grown in tumour-bearing animals.

Experimental evidence shows that normal post-mitotic cells continuously release a tissue-specific chalone which depresses cell proliferation in the mitotically active precursor cells of the same cell lineage; so far some fifteen different chalone systems have been reported in the literature (for recent reviews, see Bullough, 1972; Elgjo, 1973; Houck & Daugherty, 1974; Rytömaa, 1976; Thornley & Laurence, 1975). It has also been shown that even malignant cells produce chalone which is capable of inhibiting mitotic activity both in the tumour itself and in the corresponding normal tissue, however distant it may be (Bullough & Laurence, 1968; Rytömaa & Kiviniemi, 1970; Houck, Irausquin & Leikin, 1971; Elgjo & Hennings, 1971).

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In particular, Bullough & Deol (1971) reported that the epidermal chalone escaping from the cells of the Hewitt keratinizing epidermal carcinoma of the mouse depresses mitotic activity in the ear epidermis of the host animal; the mitotic activity of the sebaceous glands, which is controlled by a different chalone, remains unaffected (Bullough & Laurence, 1970). Similarly, the growth rate of normal and leukaemic granulocytic cells, cultured in diffusion chambers in the peritoneal cavity, was found to be inhibited in rats suffering from a granulocytic leukaemia (Vilpo, Kiviniemi & Rytömaa, 1973; Ferris, LoBue & Gordon, 1973). Mastocytoma cells, HeLa cells and Ehrlich's ascites carcinoma cells were not inhibited when grown in identical conditions and, conversely, granulocytic cells were not inhibited when grown in diffusion chambers carried by mice suffering from Ehrlich's ascites carcinoma.

It has previously been shown that psoriatic and normal human skin can be successfully cultured in diffusion chambers implanted into the peritoneal cavity of the mouse (Kariniemi, 1974). The findings referred to above prompted us to investigate whether the epidermal chalone escaping from the cells of the Hewitt epidermal carcinoma inhibits human epidermal cells when cultured in diffusion chambers carried by the tumour-bearing mice. In addition, we wanted to re-investigate and extend the findings of Bullough & Deol (1971) by measuring DNA synthesis in different squamous epithelia and in several unrelated tissues in normal and tumour-bearing animals; the main aim was to clarify the specificity of action of the inhibitor(s) released from the Hewitt epidermal carcinoma.

MATERIAL AND METHODS

Experimental animals

Seven experiments, each consisting of four or five animal pairs, were made. Four of the experiments (series A) were made in the spring, and three (series B) in the autumn. The host animals were 1·5–6-month-old female and male WHT/Ht albino mice. Half of the animals suffered from a local subcutaneous epidermal carcinoma (Hewitt keratinizing epidermal carcinoma), transplanted in the back of the mouse 17–31 days before the start of each experiment, as described earlier by Hewitt (1966). By the time the experiment was started, the tumours had grown to a size of 1–4 cm in diameter; otherwise the animals appeared to be healthy. The control animals were of the same age and sex but without the tumour.

Target cells in diffusion chamber cultures

Punch biopsies (Kariniemi, 1974) 2 mm in diameter were taken from seven patients with histologically verified psoriasis, and stored in Hanks' solution with antibiotics (penicillin and streptomycin, 100 μl/ml of each) at 4°C. Psoriatic explants rather than normal skin were used in this study because they display a more equal labelling of epidermal cells in the diffusion chamber cultures (Kariniemi, 1974). The tissue pieces were halved; one half was used for the culture in a tumour-bearing animal and the other half in a control mouse. Mouse bone marrow cells were used as the non-epidermal control tissue. These cells were obtained from adult WHT/Ht albino mice by carefully crushing the femora in Hanks' solution with antibiotics and collecting the cells in the supernatant.

Diffusion chamber technique

Diffusion chambers were made of acryl rings and Millipore filters as described earlier (Vilpo, 1972; Kariniemi, 1974). Two chambers (one without the lid of the ring) were cemented together and sterilized in an autoclave at 110°C for 10 min. Before implantation, two pieces of psoriatic skin were placed in the unfinished chamber, which was then completed by cementing another Millipore filter to form the lid of the ring. The other chamber was filled through a hole in the acryl ring by injecting 10^6

mouse bone marrow cells in 100 μl of Hanks' solution into the chamber. The hole was closed with a nylon thread and Millipore filter cement. The chamber pairs were then implanted into the peritoneal cavity of the tumour-bearing and control mice. All operations were made between 2 and 3 p.m.

The chambers were recovered on the 3rd or 4th day after implantation. 20 μCi of ^{3}H-thymidine (methyl-^{3}H, spec. act. > 10 Ci/mmol, The Radiochemical Centre, Amersham, England) was injected into each mouse intraperitoneally 1 h before killing the animal. To avoid diurnal variation between experimental and control animals, the mice were always killed in pairs. However, for practical reasons half of the pairs were killed at 10 a.m. and the other half at 1 p.m.; no distinct difference was observed between these groups.

Samples and analyses

In addition to the diffusion chamber cultures, the following samples were taken from the tumour-bearing and control mice at the termination of each experiment: pieces of ear, back and foot skin, tongue and jejunum; both femora; both corneas; spleen; thymus; inguinal lymph nodes.

Psoriatic explants, and pieces of ear, back and foot skin, tongue and jejunum were fixed in 10% neutral formalin, processed by ordinary histological techniques, sectioned at 4 μm, covered by stripping film (Kodak AR-10), exposed for 2 weeks (psoriatic samples for 5 days), processed, and stained with Harris' haematoxylin. The corneas used for the determination of mitotic indices were fixed in 95% alcohol, stained with orcein and spread on glass as described by Vasama (1961); the corneas used for autoradiography were processed in the same way as the other tissue pieces.

Both femora, carefully cleansed from soft tissues, and spleen, thymus, and lymph nodes were dried overnight at 80°C, weighed, and dissolved in Soluene (Packard Co.) at 55°C. After adding an ordinary toluene-based scintillation liquid to the samples, they were counted in a Packard Tri-Carb liquid scintillation counter for 1 min with a relative counting error of less than ±5%. The results were expressed as counts/min per mg of dry tissue or femur.

Diffusion chamber cultures initiated with bone marrow cells were shaken in 0·5% pronase solution for 70 min, the cells were collected by a pipette and counted haemocytometrically after crystal violet staining as described earlier (Vilpo *et al.*, 1973). Autoradiograms were then made of the cytocentrifuge (Shandon) preparations.

Labelling indices for the psoriatic skin were determined from the autoradiograms (identity sealed) by counting 100 cells per section in the three lowest layers of the epidermis at the cut edges of each skin piece; a total of 1000 epidermal cells was scored from each explant. The number of labelled cells in mouse epidermis was determined by scoring the cells along 10 mm of basal membrane in the tongue and in the interfollicular epidermis of the ear, foot, and back skin. To avoid regional differences in the labelling indices, the cells were counted from the transverse sections of the tongue and the ear. Labelling indices for jejunum, sebaceous glands, cornea, and bone marrow were determined by scoring 200–1000 cells. Cells with five or more grains were recorded as positive; the background was usually less than one grain per cell and the average grain count of the labelled cells was about 35; the lowest average grain count (about 10) was in the ear epidermis and the highest (about 100) in the bone marrow cells.

RESULTS

Labelling indices for the different squamous epithelia in the tumour-bearing and control mice are given in Table 1.

They show that cell proliferation was significantly inhibited in all these tissues in the mice bearing the Hewitt epidermal carcinoma. The results also show that the effect was different in different squamous epithelia; this suggests that the inhibitor escaping from the epidermal carcinoma does not

TABLE 1. Cell proliferation in different squamous epithelia in tumour-bearing (Hewitt epidermal carcinoma) and control mice

Exp. series	Group	N	No. of labelled cells/10 mm of the basal membrane				Cornea	
			Ear	Back skin	Foot skin	Tongue	Mitotic index (%)	Labelling index (%)
A	Control	17	27·1 ± 5·2	32·1 ± 2·3	33·7 ± 2·8	281 ± 23	—	—
	Tumour	17	4·3 ± 0·9	15·1 ± 2·8	16·5 ± 2·8	227 ± 18	—	—
	Inhibition (%)		84†	53†	51†	19*		
B	Control	11	8·3 ± 1·3	25·6 ± 2·4	43·6 ± 4·9	329 ± 34	2·2 ± 0·3	5·1 ± 0·2‡
	Tumour	11	2·1 ± 0·7	11·9 ± 2·4	17·1 ± 4·0	239 ± 25	1·6 ± 0·2	4·5 ± 0·9§
	Inhibition (%)		75†	53†	60†	27*	27	12

* $P<0·05$; † $P<0·001$; ‡ N = 4; § N = 3.

The values give mean ± s.e. for the relative number of labelled cells (^{3}H-TdR); N is the number of animals studied. A and B refer to two series of experiments made in spring and autumn, respectively.

reach the same concentration in the different regions or, alternatively, that different epithelial cells are not equally responsive to the inhibitor.

Cell proliferation in the sebaceous glands of the ear and in the intestinal epithelium was only slightly, if at all, inhibited in the mice bearing the Hewitt carcinoma; cell proliferation in the bone marrow (i.e. both granulopoiesis and erythropoiesis) was clearly not affected at all (Table 2). However, incorporation of ^{3}H-TdR into the spleen, and possibly also into the lymph nodes, was depressed in the tumour-bearing mice. The thymus did not display any inhibition in terms of counts/min per mg of dry tissue, but it must be pointed out that the thymuses were definitely smaller in size in the tumour-bearing mice than in the controls. It thus seems that the lymphoid tissue in general is affected in mice suffering from the Hewitt carcinoma, but this may simply be due to non-specific stress rather

TABLE 2. Incorporation ^{3}H-TdR into non-epidermal tissues in mice with and without the Hewitt epidermal carcinoma

Exp. series	Group	N	Sebaceous glands	Small intestine	Bone marrow	Spleen	Thymus	Lymph nodes
			Labelling index (%)	Labelling index (%)	cpm/ femur	cpm/mg dry tissue	cpm/mg dry tissue	cpm/mg dry tissue
A	Control	17	2·9 ± 0·3	37·0 ± 1·0	13381 ± 1002	n/d‡	n/d‡	n/d‡
	Tumour	17	2·6 ± 0·3	33·0 ± 1·5	14599 ± 1282	n/d‡	n/d‡	n/d‡
	Inhibition (%)		10	10*	—			
B	Control	11	1·6 ± 0·2	35·0 ± 1·8	13875 ± 1767	4138 ± 585	502 ± 75	4076 ± 507
	Tumour	11	1·3 ± 0·2	33·5 ± 1·3	16578 ± 744	2159 ± 369	788 ± 251	3023 ± 238
	Inhibition (%)		19	4	—	48†	—	26*

* $P<0·05$; † $P<0·01$; ‡ not determined.

The values give mean ± s.e. for each group of mice (cf. Table 1).

TABLE 3. Cell proliferation in psoriatic explants and in mouse bone marrow cultured in diffusion chambers carried by tumour-bearing (Hewitt epidermal carcinoma) and control mice

Exp. series	Group	N	Psoriatic explants	Bone marrow cultures		
			Labelling index	Total cell count*	Lab. index (all cells)	Lab. index (myeloid cells)†
A	Control	17	15·1 ± 1·3	96 ± 5	40·7 ± 1·2	48·9 ± 1·5
	Tumour	17	11·8 ± 0·9	103 ± 9	28·6 ± 2·1	43·7 ± 2·2
	Inhibition (%)		22‡	—	30§	10‡

* Scaled to a common arbitrary level by denoting the mean for each experiment at 100, in order to eliminate systematic differences in the cell count level existing between different experiments; † Definite granulocytic precursors, as judged from nuclear morphology (ring-nucleated cells); ‡ $P < 0{\cdot}05$; § $P < 0{\cdot}01$.

The values give mean ± S.E. for each group of mice (N = number of animals studied).

than to the excess of epidermal chalone (it is generally known that lymphocytes are highly sensitive to the action of corticosteroids).

The results obtained from the diffusion chamber cultures are shown in Table 3. Results from the experimental series B have been omitted, because, owing to an unknown technical failure, no cell growth occurred in several control and experimental mice. It appears that the labelling index for the epidermal cells in the psoriatic explants was reduced in the tumour-bearing mice, but clearly this effect was very much smaller than that seen in the ear epidermis and in the foot and back skin of the host animals (Tables 1 and 3). Furthermore, the nature of the inhibition seen in the psoriatic explants is in doubt, because the labelling index for all cells in the cultures initiated with bone marrow cells and grown in identical conditions was also decreased, even if the cell counts did not indicate genuine growth inhibition (Table 3).

DISCUSSION

The results of this study show that the Hewitt keratinizing epidermal carcinoma of the mouse releases one or more inhibitors which depress cell proliferation in different squamous epithelia in the host animal. This finding is, in principle, identical to that reported by Bullough & Deol (1971). The inhibitory effects were about the same in the experiments made in the spring and the autumn, respectively, although cell proliferation between these two series was markedly different in the ear epidermis and, to some extent, also in the sebaceous glands (counted from the ear). Furthermore, diffusion chamber cultures failed to grow in many animals in the autumn without apparent technical reason. We cannot offer a plausible explanation for these curious seasonal differences.

There is good reason to believe that the factor responsible for the observed inhibition is the epidermal chalone (both G_1 and G_2 inhibitors may be involved) (Elgjo, 1973; Marks, 1973). At first glance, some of our results may appear to contradict this conclusion, but plausible explanations for the few seemingly discrepant findings are readily apparent. In fact, the only finding which tends to suggest non-specific inhibition is the significantly reduced uptake of ^{3}H-TdR in the spleen. However, the real meaning of this observation is not clear at all, because Marks (1973) reported that a single injection of highly purified epidermal chalone (the G_1 inhibitor) into the mouse causes a transient inhibition in the spleen, but not in any other non-epidermal tissue studied by him. It is thus possible that for some odd reason spleen may be responsive to the epidermal chalone or, alternatively, that spleen cells are particularly sensitive even to minor stress.

It is likely that the tumour-bearing mice are stressed more than their normal counterparts; this is supported by the reactions seen in the lymphoid tissue in general. Thus, the incorporation of ^{3}H-TdR into lymph node lymphocytes seemed to be suppressed, although the effect was not statistically highly significant ($P<0.05$), and the thymuses were smaller in size. It may be worth noting, however, that cell proliferation in the remaining thymus tissue was not inhibited in the tumour-bearing mice, suggesting that the reduction in the thymus size was based on cell loss rather than on decreased cell production.

It may seem that the inhibition of the different epithelia in the tumour-bearing mice could merely be explained in terms of stress rather than excess chalone. This is not the case, however; for instance, the effect of stress hormones on cell proliferation is tissue non-specific in nature. Thus, in accordance with earlier findings (Bullough & Deol, 1971), sebaceous glands were not significantly affected in the presence of the tumour, although both the sebaceous glands and the different epithelia are sensitive to stress hormones (Bullough & Laurence, 1970). Furthermore, the response of ear and tongue to injected epidermal chalone has been found to be different, the ear being more strongly inhibited than the tongue (Laurence & Randers-Hansen, 1972), whereas both epithelia respond equally well to stress hormones (Laurence & Randers-Hansen, 1971). It thus follows that the present results cannot be explained in terms of stress alone, even if stress evidently played a role in this study.

Incorporation of ^{3}H-TdR into the psoriatic explants cultured in the diffusion chambers was slightly inhibited in the tumour-bearing mice. The response was quantitatively similar to that seen in the tongue and in the cornea of the host animals, but there is some doubt whether the effect is explicable in terms of epidermal chalone action. The uncertainty arises from the observation that the labelling index for the bone marrow cultures grown in identical conditions was similarly reduced, which suggests that a non-specific factor was responsible for both inhibitions. However, as the bone marrow of the host animal itself was clearly unaffected by the epidermal carcinoma, the reduced labelling index for the bone marrow cells cultured in diffusion chambers might be caused by the granulocyte chalone possibly present in the peritoneal fluid in the tumour-bearing animals (some tumour animals had in fact visible ascites containing granulocytes).

Regardless of the real cause of the decreased labelling indices of the cultured cells, it is clear that cell proliferation in the psoriatic epidermis was not strongly inhibited in the mice bearing the Hewitt epidermal carcinoma. Superficially, this finding is at variance with the observations made on diffusion chamber cultures of granulocytic cells in chloroma-bearing rats (Vilpo *et al.*, 1973; Ferris *et al.*, 1973). However, the difference may be explicable in terms of different physico-chemical properties of the chalones: the small-molecular-weight granulocyte chalone (MW<4000 daltons) (Rytömaa & Kiviniemi, 1968; Paukovits, 1973; Lord *et al.*, 1974) may well enter the peritoneal cavity more readily than the much larger epidermal chalone(s) (MW >30000 daltons) (Hondius-Bolding & Laurence, 1968; Marks, 1973). It was also observed by Bullough & Laurence (1960) that the diffusion range of the epidermal chalone in the tissue is only about 1 mm.

On the whole, therefore, the results of the present study are in agreement with the idea that the Hewitt keratinizing epidermal carcinoma releases large quantities of epidermal chalone, which then leads to a reduction in cell proliferation in the squamous epithelia of the host animal even in distant areas. Non-epidermal tissues, such as sebaceous glands, intestinal epithelium, granulocytic precursors, erythroid cells, and thymic lymphocytes, are not affected by this substance, indicating specificity of action. All squamous epithelia do not respond equally well to the circulating epidermal chalone, perhaps owing to regional differences in the local concentration or to some sub-specificity in the cell responsiveness (Laurence & Randers-Hansen, 1972). The finding that psoriatic human epidermal cells cultured in diffusion chambers were not decisively inhibited by the circulating epidermal chalone may be explicable in similar terms.

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REFERENCES

Bullough, W.S. (1972) The control of epidermal thickness. Parts 1 and 2. *British Journal of Dermatology*, **87**, 187, 347.

Bullough, W.S. & Deol, J.U.R. (1971) Chalone-induced mitotic inhibition in the Hewitt keratinising epidermal carcinoma of the mouse. *European Journal of Cancer*, **7**, 425.

Bullough, W.S. & Laurence, E.B. (1960) The control of epidermal mitotic activity in the mouse. *Proceedings of the Royal Society, B*, **151**, 517.

Bullough, W.S. & Laurence, E.B. (1968) Control of mitosis in rabbit V×2 epidermal tumours by means of the epidermal chalone. *European Journal of Cancer*, **4**, 587.

Bullough, W.S. & Laurence, E.B. (1970) Chalone control of mitotic activity in sebaceous glands. *Cell Tissue Kinetics*, **3**, 291.

Elgjo, K. (1973) Epidermal chalone: cell cycle specificity of two epidermal growth inhibitors. *National Cancer Institute Monograph*, **38**, 71.

Elgjo, K. & Hennings, H. (1971) Epidermal chalone and cell proliferation in a transplantable squamous cell carcinoma in hamsters. I. *In vivo* results. *Virchows Archiv: Abteilung B: Zellpathologie*, **7**, 1.

Ferris, P., LoBue, J. & Gordon, A.S. (1973) Possible feedback inhibition of leukemic cell growth: kinetics of Shay chloroleukemia grown in diffusion chambers and intraperitoneally in rodents. In: *Humoral Control of Growth and Differentation*, Vol. I, p. 213. Academic Press, New York.

Hewitt, H.B. (1966) The effect on cell survival of inhalation of oxygen under high pressure during irradiation *in vivo* of a solid mouse sarcoma. *British Journal of Radiology*, **39**, 19.

Hondius Boldingh, W. & Laurence, E.B. (1968) Extraction, purification and preliminary characterization of the epidermal chalone: A tissue-specific mitotic inhibitor obtained from vertebrate skin. *European Journal of Biochemistry*, **5**, 191.

Houck, J.C. & Daugherty, W.F. (1974) *Chalones: a Tissue-Specific Approach to Mitotic Control.* Medcom Press, New York.

Houck, J.C., Irausquin, H. & Leikin, S. (1971) Lymphocyte DNA synthesis inhibition. *Science*, **173**, 1139.

Karinıemi, A-L. (1974) Culture of psoriatic and uninvolved human skin in diffusion chambers in mice. *Journal of Investigative Dermatology*, **63**, 388.

Laurence, E.B. & Randers-Hansen, E. (1971) An *in vivo* study of epidermal chalone and stress hormones on mitosis in tongue epithelium and ear epidermis of the mouse. *Virchows Archiv; Abteilung B: Zellpathologie*, **9**, 271.

Laurence, E.B. & Randers-Hansen, E. (1972) Regional specificity of the epidermal chalone extracted from two different body sites. *Virchows Archiv; Abteilung B: Zellpathologie*, **11**, 34.

Lord, B.I., Cercek, L., Cercek, B., Shah, G.P., Dexter, T.M. & Lajtha, L.G. (1974) Inhibitors of haemopoietic cell proliferations?: Specificity of action within the haemopoietic system. *British Journal of Cancer*, **29**, 168.

Marks, F. (1973) A tissue-specific factor inhibiting DNA synthesis in mouse epidermis. *National Cancer Institute Monograph*, **38**, 79.

Paukovits, W.R. (1973) Granulopoiesis inhibiting factor: demonstration and preliminary chemical and biological characterization of a specific polypeptide (chalone). *National Cancer Institute Monograph*, **38**, 147.

Rytömaa, T. (1976) The chalone concept. In *International Review of Experimental Pathology*, Vol. XVI, in press. Academic Press, New York.

Rytömaa, T. & Kiviniemi, K. (1968) Control of granulocyte production. I. Chalone and antichalone, two specific humoral regulators. *Cell Tissue Kinetics*, **1**, 329.

Rytömaa, T. & Kiviniemi, K. (1970) Regression of generalized leukaemia in rat induced by the granulocytic chalone. *European Journal of Cancer*, **6**, 401.

Thornley, A.L. & Laurence, E.B. (1975) The present state of biochemical research on chalones. *International Journal of Biochemistry*, **6**, 313.

VASAMA, R. (1961) The diurnal rhythm of mitotic activity in the corneal epithelium of mice. *Annales Universitatis Turkuensis* Ser. A **II**, 29.

VILPO, J.A. (1972) Proliferation, survival and malignancy of chloroleukaemia cells grown in diffusion chambers *in vivo*. *Annales Medicinae Experimentalis et Biologiae Fenniae*, **50**, 168.

VILPO, J.A., KIVINIEMI, K. & RYTÖMAA, T. (1973) Inhibition of granulopoiesis by endogenous granulocyte chalone studied with the diffusion chamber technique. *European Journal of Cancer*, **9**, 515.

British Journal of Dermatology (1976) **94**, 523.

In vitro lymphocyte stimulation by wart antigen in man

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SUMMARY

The lymphocyte transformation test was used to study cell-mediated immunity to wart antigen in patients with active wart infections, in patients with past history of warts and in control subjects. A common pattern was established with wart antigen; a control baseline, followed by a rise of ^{14}C thymidine uptake in the 'active' group and a further elevation in the 'past history' group. Five patients with active warts were followed up and re-examined at various intervals after successful wart resolution; all except one responded to wart antigen by increased DNA synthetic activity after the wart resolution. This increased response was specific for wart antigen and could still be detected 6 months after wart resolution.

Recent *in vivo* and *in vitro* investigations have indicated that cell-mediated immune responses play a part in the resolution of human wart virus infections (Morison, 1974, 1975). The *in vitro* studies have used the leukocyte migration inhibition test to assess the responses. The present work was undertaken to broaden the study by using the lymphocyte transformation test as an alternative system.

MATERIALS AND METHODS

Patients

Eighteen patients (9 males and 9 females) 17–65 years of age were investigated and they were divided into two groups. The first group contained seven patients with active warts of varying types, which had been present for different periods (from 6 months to 6 years). Children and patients with ano-genital warts were excluded from the study. Blood samples were taken prior to any treatment. The second group consisted of eleven patients with a past history of warts (up to 5 years). Furthermore, five patients were investigated on two occasions; at the time of initial presentation and at various intervals (up to 6 months) after the resolution of warts. Treatment consisted of paint (formalin 20%, salicylic acid 20% in spirit) applied daily, which in one case was followed by cryotherapy using carbon dioxide.

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Seven healthy control subjects (4 males and 3 females) 19–50 years of age were also examined. None of the controls gave a history of warts within the past 10 years, although the presence of warts in childhood could not be entirely excluded.

Antigen

The wart antigen (WA) was prepared as described in detail previously and the preparation contained 10^{12} virus particles per ml (Morison, 1974). The stock suspension of the antigen was diluted to 10^{-2}, 10^{-3} and 10^{-4}, and 0·1 ml of these dilutions was added per 1 ml of culture. The wart antigen was also heated at 60°C for 2 h and this preparation was compared with untreated antigen to exclude the possible cytotoxicity of live wart virus on lymphocytes. Preservative-free PPD was used at an optimal concentration of 10 μg per ml of culture (Ivanyi & Lehner, 1974).

Lymphocyte transformation test

Lymphocytes were isolated from heparinized blood by density gradient centrifugation using a mixture of 'Ficoll-Triosil' (Ivanyi, Lehner & Burry, 1973). Cultures were prepared, harvested and evaluated as described previously (Ivanyi & Lehner, 1970). The results of lymphocyte stimulation are presented in terms of the stimulation index (SI), which is the ratio of ^{14}C thymidine uptake between antigen and saline stimulated cultures, and also as cpm/10^6 live lymphocytes placed in the cultures.

RESULTS

The dose response to various dilutions of untreated and heat-inactivated WA was tested in five patients and the optimal dilutions were found to be 10^{-4} per ml of culture (Fig. 1). The mitogenic potentials of untreated and heat-inactivated WA preparations were similar (mean SI of 4·4 and 3·9).

Lymphocytes from controls, from patients with active warts and from patients with a past history of warts were cultured in the presence of untreated and heat-inactivated WA (Table 1). A common pattern was established with both antigens; a control baseline (mean SI of 1·8 and 1·6), followed by a rise of ^{14}C thymidine uptake in the 'active group' (mean SI of 2·7 and 2·4) and a further elevation in the 'past history' group (mean SI of 4·8). The results were further classified according to the values of SI into three groups: negative ($SI < 2$), mild (SI 2·0–4·0) and strong ($SI > 4·0$) stimulation (Table 2).

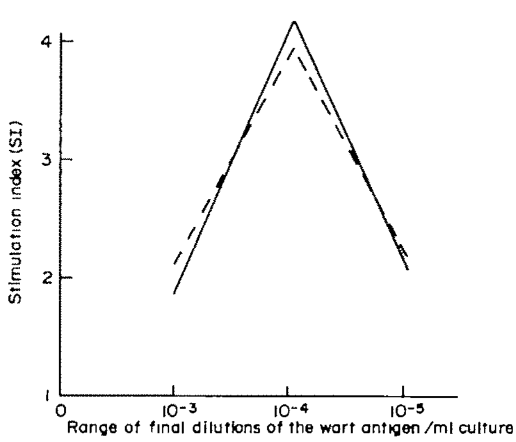

FIGURE 1. Dose response to untreated and heat-inactivated wart antigen in five patients. ——— Untreated wart antigen; – – – – heat-inactivated wart antigen.

TABLE 1. Comparison of lymphocyte stimulation induced by untreated and heat-inactivated wart antigen in patients and controls

Group	No. of subjects	Saline mean cpm	Stimulation index			
			WA untreated (mean ± s.e.)		WA heat-inactivated (mean ± s.e.)	
Controls	7	550	1·8	0·19	1·6	0·19
Active warts	7	551	2·7	0·28	2·4	0·18
Past history of warts	11	638	4·8	1·02	4·8	0·92

WA = wart antigen; cpm = counts per minute.

TABLE 2. The frequency and intensity of lymphocyte stimulation in patients and controls

Group	No. of patients	SI	WA untreated no. of responders	WA heat-inactivated no. of responders
Controls	7	Neg. < 2·0	5	5
		Mild 2–4	2	2
		Strong > 4	0	0
Active warts	7	Neg. < 2·0	1	1
		Mild 2–4	6	6
		Strong > 4	0	0
Past history of warts	11	Neg. < 2·0	2	2
		Mild 2–4	3	2
		Strong > 4	6	7

WA = wart antigen; SI = stimulation index.

TABLE 3. Comparison of lymphocyte stimulation before and after wart resolution in five patients

Patient	Interval between (months)	cpm							
		Untreated WA		Heat-inactivated WA		PPD		Saline	
		Before	After	Before	After	Before	After	Before	After
1	2	1324	5742	1087	5039	2979	3164	473	586
2	4	965	1476	1350	1476	2250	2398	643	615
3	5	1079	2205	885	1894	2822	2399	415	421
4	6	1150	2943	1260	2929	4274	4754	548	651
5	6	2090	4530	1525	3345	4068	3483	565	693

Patients were tested before resolution of their warts and at various intervals after wart resolution. The intervals between the two measurements are presented in the table.
WA = wart antigen; cpm = counts per minute.

It is apparent that DNA synthesis of lymphocytes was increased most consistently in the 'past history' group as six and seven of eleven patients responded by strong stimulation to untreated and heat-inactivated WA respectively whereas six of seven patients from the 'active group' responded by mild stimulation. In the control group stimulation induced by WA was not detected in five of seven controls; two subjects responded by mild stimulation. This difference in response between patients and controls and between the two groups of patients was statistically significant ($P<0{\cdot}05$ and $P<0{\cdot}1$).

Five patients with active warts were followed up and re-examined at various intervals after successful wart resolution (Table 3). Four out of five patients responded to WA by increased DNA synthetic activity after the resolution of their warts ($P<0{\cdot}05$). In patient No. 2 lymphocyte stimulation induced by untreated WA was slightly increased but stimulation induced by heat-treated WA did not change. This increased response to WA after wart resolution was specific, as the stimulation induced by unrelated antigen PPD was not affected by the presence or absence of warts.

DISCUSSION

The *in vitro* lymphocyte transformation test was used to study cell-mediated immunity to wart antigen in two groups of patients and controls. As wart antigen contains live virus which could be cytotoxic to peripheral blood lymphocytes, a heat-inactivated preparation was also tested. The mitogenic potentials of both untreated and heat-treated preparations were similar, suggesting that viral cytotoxicity was not important in this system. Wart antigen induced *in vitro* a proliferative response of lymphocytes from patients with 'active warts' and those with 'past history' of warts. Lymphocytes from 71% of control subjects were not stimulated by wart antigen and mild stimulation was detected in 28% of subjects. The highest level of stimulation was found in the 'past history' group as 54% of patients responded to WA by strong stimulation. Strong stimulation was not detected in the 'active group' although 85% of patients responded by mild stimulation. It was interesting to find that lymphocytes from four out of five patients revealed a change to strong *in vitro* stimulation after wart resolution. This increased DNA synthetic activity could still be detected 6 months after wart resolution. This differs from the previous finding that cell-mediated immunity tested by *in vitro* leukocyte migration inhibition lasted less than 3 months after wart resolution (Morison, 1974) and may be explained by the higher sensitivity of the lymphocyte transformation test.

These and previous results (Morison, 1974, 1975) imply a role for cell-mediated immunity in the recovery and protection from wart infection. It has also been shown that the inflammatory changes in spontaneously involuting plane warts include intense mononuclear cell infiltration in the dermis, extending into a damaged overlying epidermis, with the absence of polymorphonuclear leukocytes; this could indicate the importance of a cell-mediated immune response (Tagami *et al.*, 1974). The increased incidence of warts in patients with T-cell immunodeficiencies (Morison, 1975) and during drug immunosuppressive therapy (Spencer & Andersson, 1970) supports this view. However, it has also been reported that the risk of recurrence in patients with spontaneously regressed warts was three times higher than the incidence of warts in subjects without a history of warts (Massing & Epstein, 1963). It seems that the balance between cellular and humoral immunity is of importance; it has been shown in another system that *in vitro* cytotoxicity of Sindbis virus-sensitized lymphocytes could be blocked by Sindbis virus-immune serum (McFarland, 1974). The blocking effect of sera in various *in vitro* assays of cell-mediated immunity was also demonstrated in allograft, tumour and autoimmunity (Hellström & Hellström, 1970; Hellström, Hellström & Allison, 1971; Jagarlamoody *et al.*, 1971; Glancy, 1972) and it was suggested that IgG antibody was responsible for the blocking activity (Gatti, Yunis & Good, 1973). As rapid healing of warts was found to be associated with the presence of complement-fixing IgG antibodies (Pyrhönen & Johansson, 1975) the reported risk of recurrence in some patients might be explained on this basis.

REFERENCES

GATTI, R.A., YUNIS, E.J. & GOOD, R.A. (1973) Characterisation of a serum inhibition of MLC reactions. *Clinical and experimental Immunology*, **13**, 427.

GLANCY, R. (1972) Cellular immunity to autologous platelets and serum-blocking factors in idiopathic thrombocytopenic purpura. *Lancet*, **i**, 6.

HELLSTRÖM, K.E. & HELLSTRÖM, I. (1970) Immunological enhancement as studied by cell culture techniques. *Annual Review of Microbiology*, **24**, 373.

HELLSTRÖM, I., HELLSTRÖM, K.E. & ALLISON, A.C. (1971) Neonatally induced allograft tolerance may be mediated by serum-born factors. *Nature (London)*, **230**, 49.

IVANYI, L. & LEHNER, T. (1970) Stimulation of lymphocyte transformation by bacterial antigens in patients with periodontal disease. *Archives of Oral Biology*, **15**, 1089.

IVANYI, L. & LEHNER, T. (1974) Stimulation of human lymphocytes by B-cell mitogens. *Clinical and experimental Immunology*, **18**, 347.

IVANYI, L., LEHNER, T. & BURRY, H.C. (1973) The response of synovial fluid lymphocytes to T and B stimulants *in vitro. Immunology*, **25**, 905.

JAGARLAMOODY, S.M., AUST, J.C., TEW, R.H. & MCKHAN, C.F. (1971) *In vitro* detection of cytotoxic cellular immunity against tumour-specific antigens by radio-isotopic technique. *Proceedings of the National Academy of Science (Washington)*, **68**, 1346.

MCFARLAND, H.F. (1974) *In vitro* studies of cell-mediated immunity in an acute viral infection. *Journal of Immunology*, **113**, 173.

MASSING, A.M. & EPSTEIN, W.L. (1963) The natural history of warts. *Archives of Dermatology*, **87**, 306.

MORISON, W.L. (1974) *In vitro* assay of cell-mediated immunity to human wart antigen. *British Journal of Dermatology*, **90**, 531.

MORISON, W.L. (1975) Viral warts, herpes simplex and herpes zoster in patients with secondary immune deficiencies and neoplasms. *British Journal of Dermatology*, **92**, 625.

PYRHÖNEN, S. & JOHANSSON, E. (1975) Regression of warts. An immunological study. *Lancet*, **i**, 592.

SPENCER, E.S. & ANDERSON, H.K. (1970) Clinically evident, non-terminal infections with herpes virus and wart virus in immuno-suppressed renal allograft recipients. *British Medical Journal*, **iii**, 251.

TAGAMI, H., OGINO, A., TAKIGAWA, M., IMAMURA, S. & OFUJI, S. (1974) Regression of plane warts following spontaneous inflammation. *British Journal of Dermatology*, **90**, 147.

British Journal of Dermatology (1976) **94**, 529.

Identification of subpopulations of lymphocytes and macrophages in the infiltrate of lichen planus lesions of skin and oral mucosa

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SUMMARY

The mononuclear cells of the infiltrate in lichen planus lesions of skin and oral mucosa have been identified by detection of their membrane receptors in frozen-tissue sections. By this method, the infiltrate would appear to consist of predominantly T lymphocytes, some macrophages and only a few B cells. The pathogenesis of lichen planus is discussed in the light of these findings.

Recent investigations of lichen planus have tended to concentrate on the changes in the epidermis of the lesion. The accompanying mononuclear cell infiltrate, composed principally of lymphocytes and macrophages as shown by microscopy, has received little attention.

In recent years it has been established that lymphocytes can be divided into two ontogenetically distinct populations with different functions. The first group, the thymus derived (T) lymphocytes, are essential for cell-mediated immune responses such as delayed hypersensitivity skin reactions or graft rejection (Miller & Mitchell, 1969), and co-operate in humoral responses. The second subpopulation of lymphocytes, B (bursa equivalent) lymphocytes, are almost certainly precursors of antibody-forming cells (Janossy *et al.*, 1973), and thus are important in humoral immunity.

The third group of cells of importance in immune reactions are macrophage cells of the histiocyte-monocyte series.

Methods for distinguishing these three groups of cells from one another have now become available, both in suspensions and in tissue sections, by detection of their membrane receptors.

In suspensions, T lymphocytes can be identified by their ability to form non-immune rosettes with untreated sheep erythrocytes (E) (Lay *et al.*, 1971). Subsequently Silveira, Mendes & Tolnai (1972) reported that rosetting could be used to localize T lymphocytes in frozen sections of human lymphoid tissue.

A proportion of B lymphocytes have a membrane receptor for the activated third component of complement (C3) in antigen–antibody (A)–complement complexes (EAC) (Dukor, Bianco & Nussenzweig, 1970). In tissue sections the distribution of B lymphocytes can be demonstrated by their binding of sheep erythrocytes sensitized with IgM antibody (A) and complement (C) IgMEAC (Silveira *et al.*, 1972).

Macrophages also possess this complement receptor but can be distinguished in frozen-tissue sections by a further receptor for the Fc domain of IgG antibodies in sheep erythrocyte–IgG antibody complexes, forming IgGEA rosettes. Under these conditions there is no adherence of these sensitized sheep blood cells to B lymphocytes (Jaffe *et al.*, 1974).

This system for identifying mononuclear cells in frozen tissue sections (Table 1) has been applied to the infiltrate of lichen planus lesions of skin and oral mucosa.

TABLE 1. Membrane receptors expressed by mononuclear cells in tissue sections

Cell	IgMEAC	IgGEA	IgMEA	E
T lymphocyte	–	–	–	±
B lymphocyte	+	–	–	–
Macrophage	+	+	–	–

MATERIALS AND METHODS

Eleven patients with lichen planus, attending the Departments of Oral Medicine or Dermatology of the Welsh National School of Medicine, were studied before any treatment. Diagnostic biopsies of a skin lesion in three patients and an oral mucosal lesion in eight were performed under local anaesthesia. Each biopsy was immediately divided approximately into halves. One half was fixed in 10% formol saline and routinely processed for light microscopic diagnosis. The other half was snap frozen in a tube of isopentane, contained in liquid nitrogen and stored, wrapped in foil, at −70°C for the membrane receptor studies.

Preparation of sensitized sheep erythrocytes for rosetting

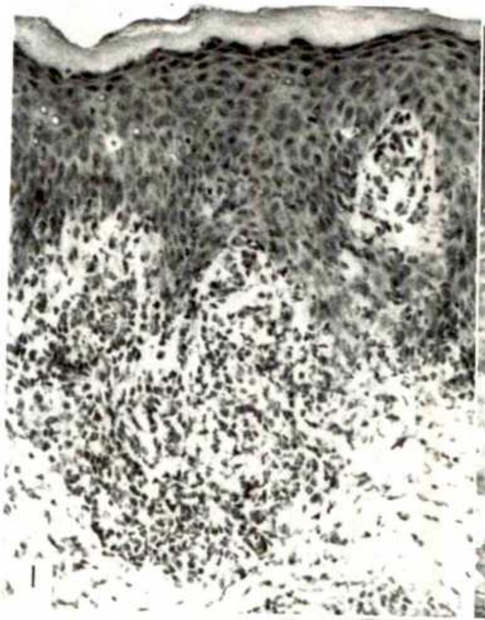
Briefly, rabbit anti-sheep erythrocyte antibodies were prepared by immunizing New Zealand white male rabbits with boiled sheep erythrocyte stroma (Frank & Gaither, 1970). The resulting high affinity antibodies were separated into IgG and IgM fractions by gel filtration on G-200 Sephadex, concentrated by ultrafiltration and then dialysed against phosphate-buffered saline (PBS).

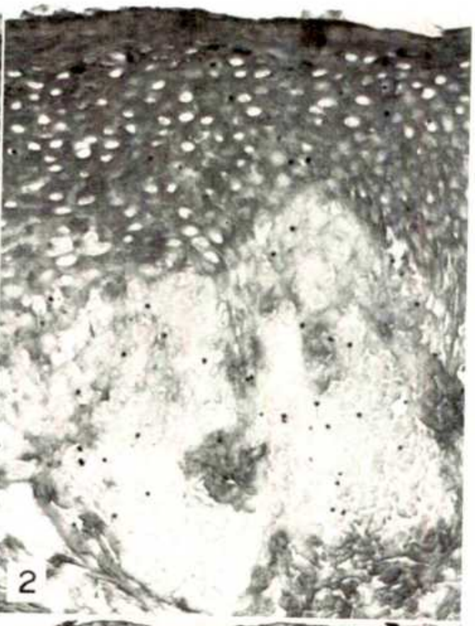
The concentration of antibody used to sensitize the sheep erythrocytes was that producing the optimal haemolysis of sheep erythrocytes at 37°C in the presence of guinea-pig complement (Wellcome Laboratories, Beckenham, Kent). In practice, a range of titres was experimented with.

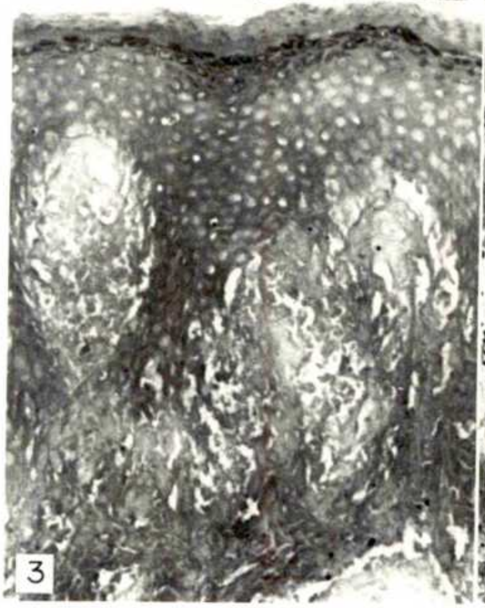
To form IgGEA and IgMEA complexes, sheep erythrocytes (E) were incubated with the optimal concentrations of IgG and IgM antibodies (A) at 37°C for 30 min as detailed previously (Edelson *et al.*, 1973). To form sheep erythrocyte-rabbit IgM antibody-complement (IgMEAC) complexes, sheep erythrocytes were incubated similarly with IgM antibody and fresh pooled mouse complement (diluted 1:10 in PBS).

Sheep erythrocyte rosetting on tissue sections of lichen planus lesions

Six μm cryostat sections of the frozen half of each lichen planus biopsy were cut, whenever possible within a month of the biopsy. The sections were allowed to dry at room temperature and then layered with IgGEA, IgMEA and IgMEAC. After 30 min at room temperature the slides were gently washed in phosphate-buffered saline (PBS) to remove any non-adherent red cells. They were then fixed in 3% glutaraldehyde and stained using an amidoblack technique (Puchtler & Sweat, 1962) which stained the overlying adherent sheep erythrocytes black to make them more obvious. A consecutive section was conventionally stained with haematoxylin and eosin.







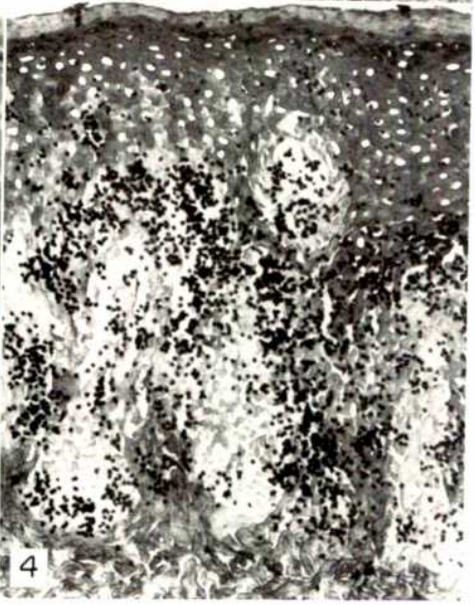


FIGURE 1. Lichen planus skin lesion (H & E, × 40).

FIGURE 2. Same lesion as in Fig. 1. Section treated with sheep erythrocytes sensitized with IgM antibody and complement. The sheep erythrocytes, stained with amidoblack adherent to sparsely scattered B lymphocytes in infiltrate (TPA, × 40).

FIGURE 3. Same lesion as in Figs 1 and 2. Section treated with sheep erythrocytes, sensitized with IgM antibody only. No sheep erythrocytes adhered to section (TPA, × 40).

FIGURE 4. Same lesion as Figs 1–3. Section treated with sheep erythrocytes, sensitized with IgG antibody. Massive adherence of sheep erythrocytes to macrophages in infiltrate (TPA, × 40).

E rosetting for T lymphocytes

Pooled sheep erythrocytes (Wellcome) in Alsever's medium, used within 1 week of bleeding, were washed three times in PBS and then resuspended in Medium 199 with 10% fetal bovine serum at a final concentration of 2% v/v. These sheep erythrocytes were layered on further frozen sections of lichen planus lesions and incubated at 37°C for 10 min in a humidity chamber and subsequently overnight at 0°C. They were then washed, fixed and stained as above. As a control, sheep erythrocytes (E) layered on further sections were incubated at 37°C throughout. Under these conditions, E rosetting in T lymphocytes does not occur (Lay *et al.*, 1971).

As a marker of activated T lymphocytes (Sheldon & Holborow, 1975), human Group O Rhesus Negative erythrocytes were substituted for the sheep erythrocytes in further sections. They were layered on sections of lichen planus lesions of two cases to date and incubated for 10 min at 37°C, then overnight at 0°C.

Normal lymphoid tissue

As a further control, sections of normal human lymph nodes and normal mouse spleen were similarly treated.

RESULTS

In Fig. 1, a lichen planus skin lesion has been sectioned and stained conventionally to show the infiltrate.

In Fig. 2, sheep erythrocytes, sensitized with IgM antibody and complement (IgMEAC), have been layered onto a consecutive frozen section of the same lesion. Sheep erythrocytes, stained black, were sparsely but uniformly adherent throughout the zone of the infiltrate as markers of B lymphocytes.

As a control, a further consecutive section treated with sheep erythrocytes coated with IgM antibody only (IgMEA) showed no adherence (Fig. 3), indicating that complement receptors of the mononuclear cells were being identified in Fig. 2.

Attempts to identify T lymphocytes by treating frozen sections of lichen planus lesions with sheep erythrocytes (E) alone have produced inconsistent results. Incubation at 37°C throughout the entire procedure was negative as a control. Attempts at identification of activated T lymphocytes using human Group O Rhesus Negative erythrocytes were positive, but only two biopsies were studied.

As particulate markers of macrophages, further sections of the lesion were treated with sheep erythrocyte-IgG antibody complexes; significant numbers of macrophages were thus labelled and were evenly distributed throughout the infiltrate (Fig. 4). These patterns of adherence of each immunological marker were essentially the same in all the skin and mucosal lesions studied.

In the normal human lymphoid tissue used as a control on the same occasion, the same IgMEAC complexes adhered predominantly into the germinal centres of the lymphoid follicle, indicating selective adherence to B lymphocyte areas (Silveira *et al.*, 1972), sparing the interfollicular areas (T cell areas). The IgGEA reagent attached to the macrophages of the medullary sinuses of the human lymph nodes and histiocytes of the red pulp of the mouse spleen. However, attempts to delineate the T cell compartments of these lymphoid tissues by E rosetting proved inconsistent and at variance with the distribution of T lymphocytes suggested by other methods (Parrott, de Sousa & East, 1966).

DISCUSSION

Evidence has been presented that skin and mucosal lesions of lichen planus are similar in that they have only small numbers of B lymphocytes uniformly dispersed through the infiltrate. Since B lymphocytes are almost certainly precursors of antibody-forming cells, this supports the findings of Lai a Fat, Suurmond & van Furth, (1973), that immunoglobulin synthesis and content of lichen planus lesions is relatively insignificant. Humoral immunity would appear to play little part in the pathogenesis of the disease therefore. As further evidence for this, no lymphoid cells with appreciable amounts of rough endoplasmic reticulum profiles, characteristic of antibody-forming cells, have been found in an electron-microscopic examination of the infiltrate (Walker, 1976, in preparation).

The role of the macrophages identified in appreciable numbers by their membrane receptors is not known. Some act as scavengers since they contain phagocytosed material such as melanin and degenerate epithelial material (El-Labban, 1972). Whether the majority of macrophages are concerned in processing of an antigen in a cell-mediated auto-immune reaction and interacting with T lymphocytes can only be speculated upon.

The majority of the mononuclear cells in the infiltrate showed neither of the membrane receptors characteristic of B lymphocytes or macrophages and by exclusion are probably T lymphocytes. Attempts to demonstrate T lymphocytes directly in sections of lichen planus lesions by their receptors for sheep erythrocytes were invalidated by the failure of this method in the normal lymphoid tissue. Chused *et al.* (1974) have also recently denied that E rosettes form on T lymphocytes in sections.

Therefore, attempts were made to demonstrate these T lymphocytes by E rosetting in cell suspensions produced by finely mincing the connective tissue of lichen planus lesions. However, only a low yield of uncontaminated viable mononuclear cells resulted and this technical problem has not yet been overcome personally.

Independent confirmation that T lymphocytes predominate, with few B cells, has just become available from Tan, Byrom & Hayes (1975), who identified the inflammatory cells extracted from lichen planus lesions with collagenase.

This conclusion that the majority of infiltrating cells are T lymphocytes with some macrophages is also consistent with the recent case reports of Saurat *et al.* (1975) and Touraine *et al.* (1975). They detailed five cases of lichen planus of skin and oral mucosa occurring in patients developing graft-versus-host reactions after receiving allogeneic bone-marrow grafts. T cells are known to be essential for such graft-versus-host reactions (Taylor, 1963).

It follows that lichen planus eruptions, whether apparently idiopathic, drug-induced or graft-versus-host reactions, may in each case represent a cell-mediated (possibly T cell) immune response to an induced antigenic change in the skin or mucosa.

The earliest event detected by electron microscopy in the lichen planus lesion is the liquefaction degeneration of the basal epithelial layer (Sarkany & Gaylarde, 1971). The inflammatory infiltrate occurs later, and following the same hypothesis, is induced by an antigenic change in the epithelium associated with the liquefaction degeneration.

In support of this, the mean nuclear labelling index of the infiltrate cells of oral lesions was significantly greater than that of normal mucosa, using *in vitro* autoradiography (Walker & Dolby, 1976, in preparation). The labelled cells were concentrated in the subepithelial zone.

In further work, attempts were made to demonstrate this antigen directly *in vitro* by incubating tissue extracts of lichen planus lesions or adjacent uninvolved skin and mucosa with autologous peripheral blood lymphocytes. Lymphocyte transformation of these lymphocyte cultures and two other correlates of cell-mediated immunity were negative. However, the range of protein concentrations used for the tissue antigens was limited.

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REFERENCES

CHUSED, Y.M., HARDIN, J.A., FRANK, M.M. & GREEN, I. (1974) Identification of cells infiltrating the minor salivary glands in patients with Sjogren's Syndrome. *Journal of Immunology*, **112**, 641.

DUKOR, P., BIANCO, C. & NUSSENZWEIG, V. (1970) Tissue localization of two populations of human lymphocytes distinguished by membrane receptors. *Journal of Immunology*, **108**, 1456.

EDELSON, R.L., SMITH, R.W., FRANK, M.M. & GREEN, I. (1973) Identification of subpopulations of mononuclear cells in cutaneous infiltrates. *Journal of Investigative Dermatology*, **61**, 82.

EL-LABBAN, N.G. (1972) A study of oral lichen planus using histological and electron microscopic methods and a computer aided comparison between lichen planus and some other white lesions of the oral mucosa. *PhD Thesis*, University of London.

FRANK, M.M. & GAITHER, T. (1970) The effect of temperature on the reactivity of guinea-pig complement with IgG and IgM haemolytic antibodies. *Immunology*, **19**, 967.

JAFFE, E.S., SHEVACH, E.M., FRANK, M.M. & GREEN, I. (1974) Leukemic reticuloendotheliosis; presence of a receptor for cytophilic antibody. *American Journal of Medicine*, **57**, 108.

JANOSSY, G., SHOHAT, M., GREAVES, M.F. & DOURMASHKIN, R. (1973) The ultrastructural pattern of the response of mouse T and B cells to stimulation *in vitro*. *Immunology*, **24**, 211.

LAI A FAT, R.F.M., SUURMOND, D. & VAN FURTH R. (1973) *In vitro* synthesis of immunoglobulins secretory component and complement in normal and pathological skin and the adjacent mucous membranes. *Clinical and Experimental Immunology*, **14**, 377.

LAY, W.H., MENDES, N.F., BIANCO, C. & NUSSENZWEIG, V. (1971) Binding of sheep red blood cells to a large population of human lymphocytes. *Nature (London)*, **230**, 531.

MILLER, J.F.A.P. & MITCHELL, G.F. (1969) Thymus and antigen reactive cells. *Transplantation Review*, **1**, 3.

PARROTT, D.M.V., DE SOUSA, M.A.B. & EAST, J.J. (1966) Thymus dependent areas in the lymphoid organs of neonatally thymectomized mice. *Journal of Experimental Medicine*, **123**, 191.

PUCHTLER, H. & SWEAT, F. (1962) Amidoblack as a stain for haemoglobin. *Archives of Pathology*, **73**, 81.

SARKANY, J. & GAYLARDE, P.M. (1971) Ultrastructural and light microscopic changes of the epidermo–dermal junction. *Transactions of St John's Hospital Dermatological Society*, **56**, 48.

SAURAT, J.H., GLUCKMAN, E., BUSSEL, A. & DIDIER-JEAN, L. (1975) Les éruptions lichéniennes après greffe de moelle allogénique. *La Nouvelle Presse Medicale*, **4**, 745.

SHELDON, P. & HOLBOROW, E.J. (1975) Human erythrocyte rosette formation with mitogen-stimulated human lymphocytes—a marker for demonstration of activated T cells. *Journal of Immunogical Methods*, **7**, 379.

SILVEIRA, N.P.A., MENDES, N.F. & TOLNAI, M.E.A. (1972) Tissue localization of two populations of human lymphocytes distinguished by membrane receptors. *Journal of Immunology*, **108**, 1456.

TAN, R.S.-H., BYROM, N.A. & HAYES, J.P. (1975) A method of liberating living cells from the dermal infiltrate: studies on skin reticuloses and lichen planus. *British Journal of Dermatology*, **93**, 271.

TAYLOR, R.B. (1963) Immunological competence of thymus cells after transfer to thymectomized recipients. *Nature (London)*, **199**, 873.

TOURAINE, R., REVUZ, J., DREYFUS, B. & ROCHANT, N. (1975) Graft-versus-host reaction and lichen planus. *British Journal of Dermatology*, **92**, 589.

British Journal of Dermatology (1976) 94, 535.

Lysosomal hydrolases of the epidermis

5. VARIATION WITH DEPTH IN THE COW SNOUT

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SUMMARY

The activity of twelve acid hydrolases has been measured in serial horizontal sections of cow snout epidermis. The majority of these enzymes showed a clear peak in the basal and lower spinous cell layers; acid phosphatase, however, was found mainly in the keratinizing zone.

These findings suggest that two discrete populations of lysosomes may occur in epidermis. Those present in the lower layers possess a typical pattern of hydrolase activity and probably correspond to the 'orthodox' lysosomes observed by electron microscopy techniques, whereas those found in the keratinizing zone possess an unusual hydrolase profile and may correspond to the bodies identified morphologically as keratinosomes (Odland bodies, membrane-coating granules).

Lysosomes may be defined either in terms of morphology or of biochemistry. To date, there has been little real correlation between these two approaches when applied to epidermis; many points, such as the distribution of lysosomes within the epidermis remain equivocal.

In morphological terms, classical lysosomes seem to occur mainly in the lower layers. Thus Wolff & Schreiner (1970) stated that 'structures meeting the morphologic criteria of lysosomes' were 'usually, but not exclusively' found in basal and lower spinous cells. On the other hand, structures such as the keratinosome (Odland bodies, membrane-coating granules) have been described in the upper layers of the epidermis (Odland, 1960); these have also been regarded as lysosomes by certain authors (Wolff & Holubar, 1968; Hayward, 1974). In biochemical terms, evidence regarding the distribution of lysosomes has been available only with the use of histochemical staining techniques. Again no clear-cut answer has emerged. Certain enzymes, apparently characteristic of lysosomes, seem to be found mainly in the basal cell layer, whilst others have been reported to occur chiefly in the keratinizing zone. The histochemical literature has been extensively reviewed by Jarrett (1973a).

In an effort to clarify this situation we have examined the hydrolase profile of cow snout epidermis in relation to depth, the unusual thickness of this tissue permitting quantitative biochemical investigation of serial slices (Long, 1970). Our findings are presented below.

MATERIALS AND METHODS

Cow snouts were packed in ice and transported to the laboratory within 2 h of slaughter. Samples of whole skin of about 0·5 cm diameter were removed and serial horizontal sections of 10 μm thickness were cut using a cryostat. Usually 50–80 sections could be obtained before excessive dermal contamination was observed. In general, groups of four consecutive serial sections were used for biochemical assay, each fifth section being set aside for histological examination; in the case of arylsulphatase B and cathepsin C groups of eight sections were employed.

Each batch of sections was weighed and homogenized in 0·5 ml bovine serum albumin solution (1 mg/ml) using a 'micro-wet grinder' (Townson and Mercer) provided with an ice-jacket, and the homogenate centrifuged to yield a clear supernatant. Enzyme activity was measured in duplicate 0·1 ml samples; other than the concentrations of the epidermal extracts, the assay methods were exactly as described previously (Mier & Van den Hurk, 1975 a–c).

RESULTS

The distribution of the seven glycosidases (Fig. 1) was remarkably similar; all showed clear peaks in the basal cell layer, the activity falling to very low levels in the keratinizing zone. The esterases and peptide hydrolases, however, yielded rather different patterns (Fig. 2). Within this latter group acid phosphatase was unique in possessing maximal activity in the upper layers. Pyrophosphatase was evenly distributed across the epidermis and cathepsin C, whilst most active in the basal cells, still showed appreciable activity in the keratinizing zone.

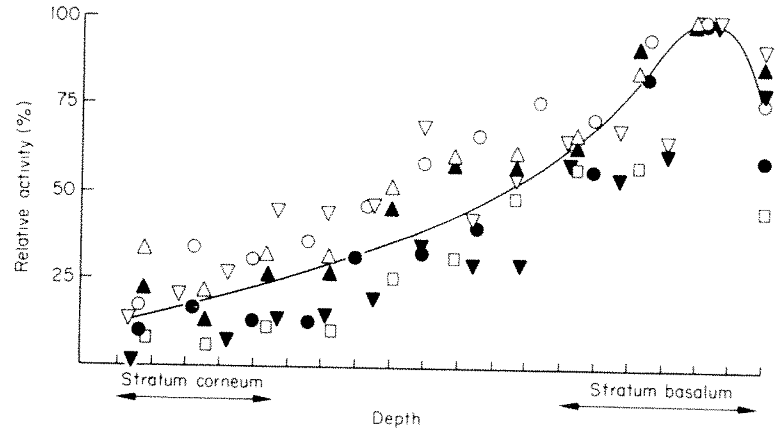

FIGURE 1. The distribution of 7 glycosidases across the epidermis of cow snout. ○—○, α-glucosidase; ●—●, β-glucosidase; △—△, α-galactosidase; ▲—▲, β-galactosidase; □—□, α-mannosidase; ▽—▽, β-N-acetylglucosaminidase; ▼—▼, β-glucuronidase.

DISCUSSION

In the previous paper in this series (Mier & Van den Hurk, 1975 d) we reported that acid phosphatase and, to a lesser extent, pyrophosphatase showed unusually high levels of activity in epidermis in

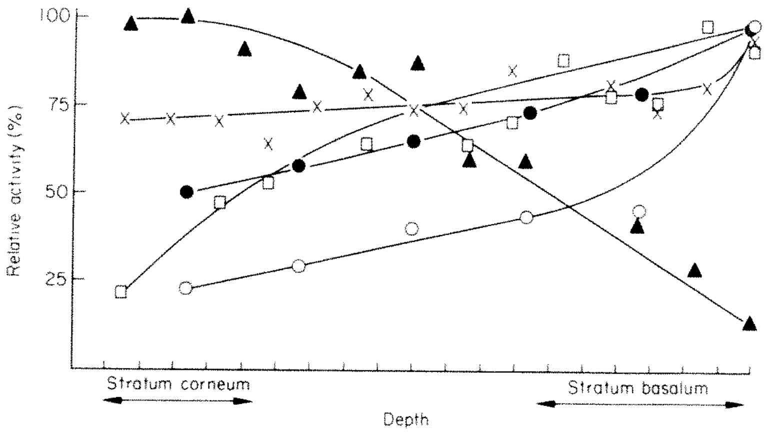

FIGURE 2. The distribution of certain ester hydrolases and peptide hydrolases across the epidermis of cow snout.
▲—▲, acid phosphatase; × — ×, pyrophosphatase; □—□, arylamidase; ○—○, arylsulphatase B; ●—●, cathepsin C.

comparison with other tissues. We now find that these same two enzymes are distributed within the epidermis in a markedly atypical manner.

A priori, two interpretations of this data are possible. On the one hand, we may imagine the hydrolase profile of the individual lysosome to change dramatically during the transit of the keratinocyte across the epithelium. On the other hand, we may suppose two distinct populations of lysosomes to exist, one occurring in the lower layers and the other in the keratinizing zone of the epidermis. Both the general body of knowledge regarding lysosomes and the morphological evidence pertaining to epidermis lead us strongly to favour the second of these alternatives.

On this assumption, we may construct separate hydrolase profiles for each population by collating the data from Figs 1 and 2. In the case of the lower layers, such a profile is quite similar to that found in many other mammalian tissues (Mier & Van den Hurk, 1975 d). Thus the conclusions of Wolff & Schreiner (1970) that 'the evolution and dynamics of lysosomes in keratinocytes conform to the general lysosome concept' can be extended to include also biochemical conformity.

The hydrolase profile of the keratinizing zone, however, is quite unique, containing an overwhelming preponderance of acid phosphatase; several enzymes, such as β-glucuronidase, which are usually regarded as 'markers' for lysosomes are virtually absent. It seems likely that this second population corresponds to the keratinosomes, since the distribution of these structures coincides with that which we find for acid phosphatase (Fig. 2), and since keratinosomes have already been shown to contain this enzyme (Wolff & Holubar, 1968; Hayward, 1974). The presence of appreciable quantities of cathepsin C is compatible with the hypothesis that these bodies are responsible for the breakdown of desmosomes (Jarrett, 1973 b), and the presence of pyrophosphatase (probably identical to phosphodiesterase IV) suggests that keratinosomes play a role in the degradation of nuclear material which accompanies the keratinization process.

REFERENCES

HAYWARD, A.F. (1974) Membrane coating granules are secondary lysosomes. *Journal of Anatomy*, **118**, 364.

JARRETT, A. (1973a) Acid hydrolases of the epidermis. In: *The Physiology and Pathophysiology of the Skin*, vol. 1, p. 182. Academic Press, London. *Idem* (1973b). Membrane coating granules, p. 198.

LONG, V.J.W. (1970) Variation in lipid composition at different depths in the cow snout epidermis. *Journal of Investigative Dermatology*, **55**, 269.

MIER, P.D. & VAN DEN HURK, J.J.M.A. (1975a). Lysosomal hydrolases of the epidermis. 1. Glycosidases. *British Journal of Dermatology*, **93**, 1. *Idem* (1975b) 2. Ester hydrolases, **93**, 391. *Idem* (1975c) 3. Peptide hydrolases, **93**, 509. *Idem* (1975d) 4. Overall profile in comparison with dermis and other tissues, **93**, 443.

ODLAND, G.F. (1960) A submicroscopic granular component in human epidermis. *Journal of Investigative Dermatology*, **34**, 11.

WOLFF, K. & HOLUBAR, K. (1968) Odland-Körper (Membrane Coating Granules, Keratinosomen) als epidermale Lysosomen: Ein elektronen-mikroskopisch-cytochemischer Beitrag zum Verhornungsprozess der Haut. *Archiv für klinische und experimentelle Dermatologie*, **231**, 1.

WOLFF, K. & SCHREINER, E. (1970) Epidermal lysosomes: electron microscopic-cytochemical studies. *Archives of Dermatology* **101**, 276.

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Subcutaneous phycomycosis in Uganda

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SUMMARY

This paper reports a further eighty cases of subcutaneous phycomycosis infection in Uganda seen between 1967 and 1974. This brings the number of reported cases from this country to over 140 and indicates an unusual frequency. The infection is more common in certain northern areas but does not appear to be confined to one ecological area. Suggestions are made as to possible factors in the initiation of this unusual infection.

Subcutaneous phycomycosis was first described in Indonesia by Joe *et al.* (1956). In the same year Raper recorded five cases of 'creeping subcutaneous granuloma' from Uganda. It was later recognized that these cases were typical of subcutaneous phycomycosis and in 1964 Burkitt, Wilson & Jelliffe reported thirty-one cases all of which were diagnosed histologically and some by culture of the organisms of the Entomophtherales group. Luyombya-Sengero (1966) published a further twenty-nine cases seen in Uganda over a 4-year period. Since 1966 a further eighty cases have been diagnosed by the central pathology service at Makerere University Medical School which suggests that there is an unusually high frequency of this condition in Uganda. The purpose of this paper is to record these eighty cases and to review the findings in the light of previous reports of this condition.

MATERIALS AND METHODS

The Pathology Department of Makerere University Medical School gives a free biopsy service to the whole of Uganda and all material received is kept and classified for retrieval. As part of a wider study, all biopsies classified as fungal infections were re-examined. In eighty cases a confirmed diagnosis of subcutaneous phycomycosis was made on the basis of the presence of characteristic hyphae in the subcutaneous tissue. There were ten other cases in which the clinical and histopathological features were compatible with this diagnosis but they were excluded because the hyphae could not be identified with certainty. Silver methenamine (Grocott) and Periodic Acid-Schiff stains were used to delineate the hyphae in many cases. In the eighty cases with a definite diagnosis the clinical information was extracted, together where possible with information on tribal origin and place of residence. Unfortunately the site of the hospital where the biopsy was taken does not always give a true indication of the patient's origin.

RESULTS

There were fifty male and thirty female patients in this series (male:female ratio of 5:3). The ages ranged from 1 to 58 years (Fig. 1). Forty-seven per cent of the cases were under 5 years and 76% under the age of 10. Only 12% were over 20 years. The duration of symptoms was given in only 42 cases and ranged from 2 weeks to 5 years with an average of 6 months.

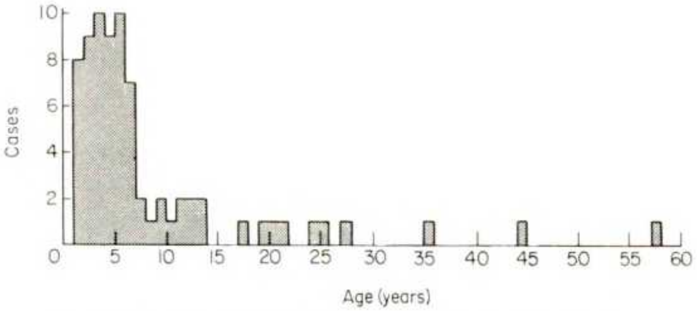

FIGURE 1. Age distribution of subcutaneous phycomycosis.

TABLE 1. The different tribes infected with subcutaneous phycomycosis in sixty-eight cases

Tribe	Langi	Ganda	Soga	Etesot	Acholi Rwanda-Rundi
No. of cases	16	15	10	9	4 each tribe

None of the other remaining tribes showed more than two cases.

The tribal distribution of the cases is shown in Table 1 and the geographical areas from which they came in Fig. 2. In assessing the significance of these findings they have to be related to the size of each tribal group and also to the overall frequency of biopsies (representing to some extent the availability of hospital facilities) in the area, e.g. the Ganda population is about 6 times the population of Langi and few biopsies (less than 10%) come from the northern areas of Uganda. On this basis sixteen cases from Lango represent a very high incidence in this region and there is a pronounced deficiency of cases from the mountainous areas of the south-west.

Subcutaneous phycomycosis usually presents as a diffuse firm mass in the subcutaneous tissues. The lesion is usually single though occasionally more than one site is involved. The site of the main lesion was recorded in seventy-five cases in this series (Table 2). Forty-nine of the cases had lesions in the area of the buttock or thigh, sometimes involving both sites or spreading onto the abdominal wall. The lesions varied in extent, occasionally involving a whole limb (Fig. 3) and necessitating amputation. While deeper structures such as fascia and muscle were occasionally involved, no disseminated lesions were ever seen and during the last 20 years no case has ever come to post mortem in Uganda.

The histopathological reaction has been described in detail and is usually characteristic. Examination of many lesions allows one to build up a picture of the probable progression of the disease. In the early stages hyphae are easily seen, often surrounded by a zone of granular eosinophilic material adjacent to the hyphal wall. In sections stained with Haematoxylin and Eosin the hyphae often appear

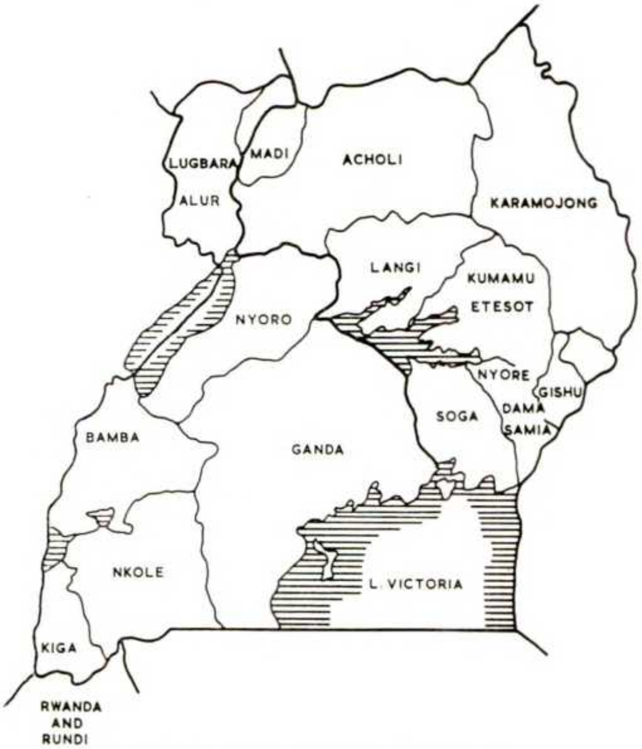

FIGURE 2. Map of Uganda showing tribes.

as hollow tubular or circular 'holes', though careful search will often reveal septa in longitudinal sections. Special stains, such as Grocott, usually reveal more hyphae than are evident in routine sections. Around the hyphae is a variable inflammatory response which may be composed predominantly of eosinophils forming eosinophilic abscesses (Fig. 4). The duration of this reaction is unknown but it is followed by the appearance of increasing number of macrophages which assume an epithelioid appearance and form distinct granulomas (Fig. 5). These may contain giant cells inside which distorted fragments of fungal hyphae may be seen (Fig. 6). At this stage there may be a more diffuse inflammatory reaction containing eosinophils, lymphocytes, plasma cells and macrophages, and there is an increasing fibroblastic response which gradually dominates the picture. In large lesions

TABLE 2. Showing the site of involvement in seventy-five cases of subcutaneous phycomycetes

Main site	No. of cases	Extension to other sites
Buttock	28	Thigh in 3
Thigh	21	Buttock in 3
Upper limb	8	Neck in 1
Leg	6	—
Trunk	6	—
Head & neck	4	Upper limb in 1
Penis	1	—
Vulva	1	—

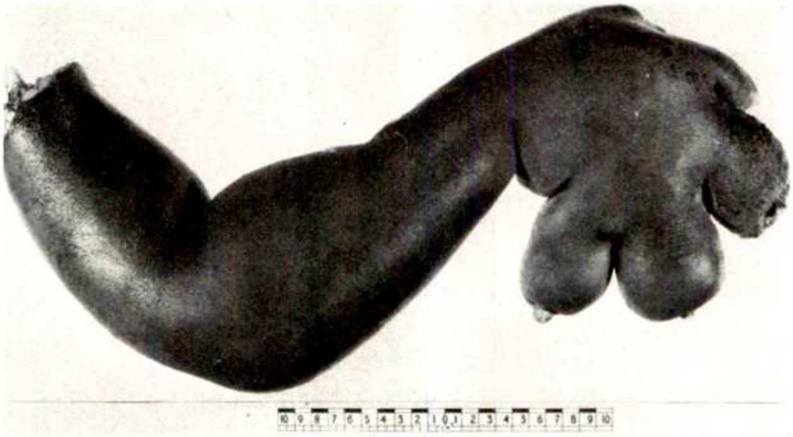

FIGURE 3. An amputated right arm diffusely infiltrated by subcutaneous phycomycosis.

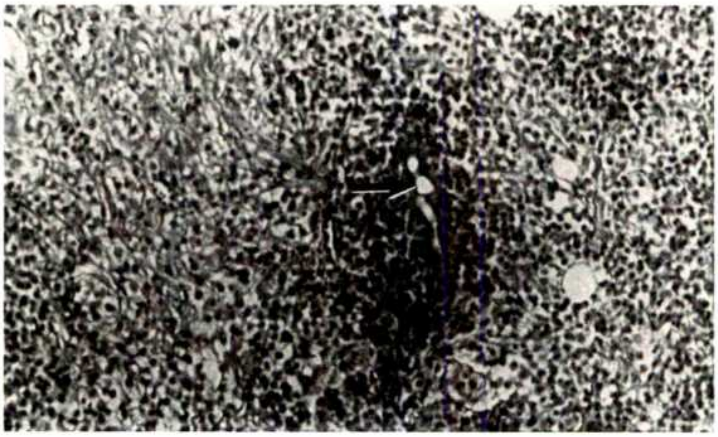

FIGURE 4. Acute inflammatory cells around a fungal hypha (arrow). (H & E, × 80.)

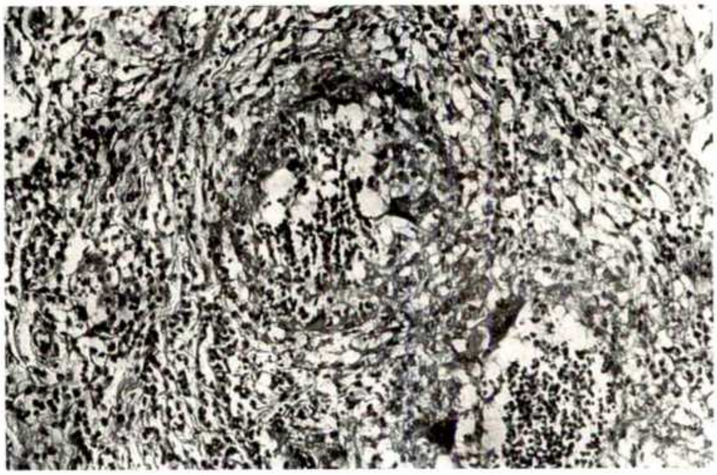

FIGURE 5. A suppurating granuloma in subcutaneous phycomycosis. (H & E, × 80.)

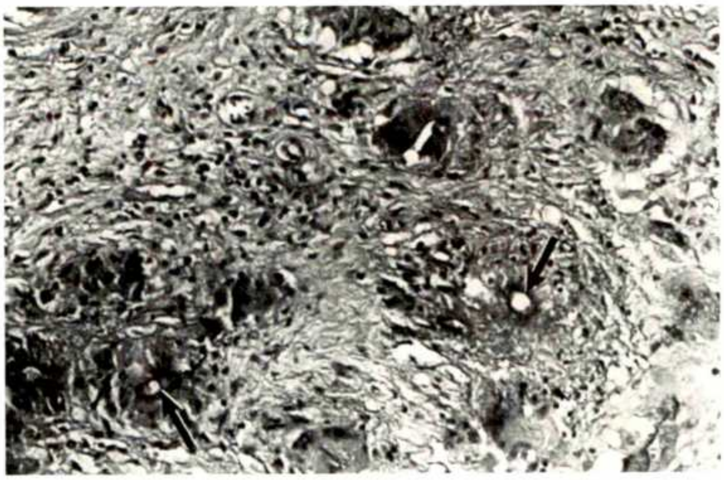

FIGURE 6. A hyphae (arrow) in giant cells in subcutaneous phycomycosis. (H & E, × 80.)

the various stages described may be seen in different parts of the same section. The dermal tissues may show some reactive changes but hyphae are rarely seen in this area and ulceration was a feature in only three of the present cases despite extensive involvement of the skin. In none of the cases was there involvement of the lymph nodes.

DISCUSSION

When taken with previous reports from Uganda (Burkitt *et al.*, 1964; Luyombya-Sengero, 1966; Hutt, 1968) this series brings the total of cases in this country to over 140 which is by far the largest series reported from anywhere in the world. In 1968 Clark summarized seventy-eight cases from the world literature, many of which had been reported from tropical Africa. In 1973 Cameron, Gatei and Bremner described nineteen cases from Kenya, two of which were fatal. Outside Africa, reports have come from Burma, India, Iraq and Indonesia (Clark, 1968). The mycology of subcutaneous phycomycosis was reviewed by Clark (1968) and it is generally accepted that the causative organism is *Basiobolus haptosporus*, an organism which morphologically resembles *Entomophorata coronata*, the cause of rhinophycomycosis.

The clinical features have been well delineated in previous papers and the diagnosis must be considered in any diffuse subcutaneous mass, particularly if it occurs in the region of the buttocks or thigh of a young child in the tropics. Although not usually associated with systemic symptoms or spread, the fungus may penetrate deep structures including blood vessels with subsequent visceral involvement (Edington, 1964; Ridley & Wise, 1965; Symmers, 1960; Cameron *et al.*, 1973). One case has been seen in Uganda in which a patient presented with an intestinal mass thought to be a tumour. At resection the tissues removed were found to contain a fungus morphologically resembling phycomycosis; it is possible that the organism was ingested with soil.

The histological features suggest the gradual development of a type IV delayed hypersensitivity reaction to the fungus characterized by granuloma formation and fibrosis. Williams (1969) using electron microscopy came to the conclusion that the perihyphal eosinophilic zone seen in earlier lesions is due to an antigen–antibody reaction similar to that seen around schistosomal ova.

Epidemiology. The 140 cases from Uganda show a wide range of geographical situations but with an undoubted high frequency in parts of the northern areas, especially Lango. Cameron *et al.* (1973) reported that eleven out of nineteen patients in Kenya were pastoral Somali or Boran, inhabiting hot dry country. The organism is widely distributed in nature and Clark (1968) has cultured it from soil and from the alimentary tract of certain reptiles. It is generally accepted that the organism gains access to the tissues by direct inoculation from vegetation or possibly mites or insects. The distribution of the lesions and the age of the patients suggest to the author that the organism may gain access to the body in connection with the act of defaecation. The affected children are unlikely to use napkins, and 'toilet leaves' would be used after a bowel action; these leaves differ in type in different areas. They may carry the organism and skin trauma is frequent in 'cleaning up' in these situations. Female children tend to be more meticulous in their habits and this might explain the sex difference. The distribution of the disease may be related either to the ecological conditions which favour fungal growth or to cultural differences such as described here which facilitate inoculation of the organism. It is of some interest that the other common disease that infects the subcutaneous tissues of Ugandans, *Mycobacterium ulcerans* infection (Buruli ulcer), is probably introduced into the tissues from special types of grasses and that differences in social and occupational behaviour between children and adults and between males and females account for differences in the distribution of the lesions in this condition. It is hoped that these observations from the large series of cases in Uganda may stimulate more interest in the epidemiology of this disfiguring condition.

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REFERENCES

Burkitt, D.P., Wilson, A.M.M. & Jelliffe, D.B. (1964) Subcutaneous phycomycosis: A review of 31 cases seen in Uganda. *British Medical Journal*, **i**, 1669.

Cameron, H.M., Gatei, D. & Bremner, A.D. (1973) Deep mycoses in Kenya. *East African Medical Journal*, **50**, 396.

Clark, B.M. (1968) The epidemiology of phycomycosis. *Systemic Mycosis* (Ed. by G.E.W.Wostenholme and Ruth Porter), pp. 179–192. Churchill, London.

Edington, G.M. (1964) Phycomycosis in Ibadan, Western Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **58**, 242.

Hutt, M.S.R. (1968) Subcutaneous phycomycosis in Uganda. *Systemic Mycosis* (Ed. by G.E.W.Wostenholme Ruth Porter), pp. 193–194. Churchill, London.

Jelliffe, D.B., Wilson, A.M.M. & Burkitt, D.P. (1962) Subcutaneous phycomycosis responding to oral therapy. *Journal of Paediatrics*, **61**, 448.

Lei Kian Joe, Njo-Injo Tjoei Eng, Pohen, A. & Van der Muelen, H. (1956) *Basidiobolus ranarum* as a cause of subcutaneous phycomyocosis in Indonesia. *Archives of Dermatology and Syphilology*, **74**, 378.

Raper, A.B. (1956) Subcutaneous granuloma of unknown aetiology. *East African Medical Journal*, **33**, 356.

Luyombya-Sengero, J.M. (1966) Fungus infections in Uganda. *Makerere Medical Journal*, **10**, 36.

Ridley, D.S. & Wise, M. (1965) Unusual disseminated infection with a phycomycetes. *Journal of Pathology and Bacteriology*, **90**, 675.

Symmers, W.St.C. (1960) Mucormycotic granuloma possibly due to *Basidiobolus ranarum*. *British Medical Journal*, **i**, 1331.

Williams, A.O. (1969) Pathology of phycomycosis due to *Antomophthora* and *Basidiobolus* species. *Archives of Pathology*, **87**, 13.

Williams, A.O., Lichtenberg, F.V., Smith, J.H. & Martinson, F.D. (1969) Ultrastructure of phycomycosis. *Archives of Pathology*, **87**, 459.

Wilson, A.M.M. (1961) Two phycomycetes isolated from subcutaneous phycomycosis in Uganda. *Nature*, **191**, 612.

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The effect of clobetasone butyrate and other topical steriods on skin thickness of the domestic pig

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SUMMARY

A new glucocorticoid, clobetasone butyrate, has been shown in patients to have good topical anti-inflammatory activity and a minimal effect on hypothalamic–pituitary–adrenal function. Among a group of topically active corticosteroids, compared in a controlled study in the domestic pig, clobetasone butyrate is shown to cause less epidermal thinning than any of the others except only 1% hydrocortisone. This evidence of a lesser atrophogenic effect may indicate further dissociation of unwanted from wanted properties in clobetasone butyrate.

The propensity to cause atrophy of the skin has not yet been shown to be separable from the topical anti-inflammatory activity of glucocorticoids.

There is no simple way of screening new compounds for this atrophogenic effect. Small laboratory animals are not easy to treat with repeated local applications, and their skin is very different from that of the human. The young Large White domestic pig has skin and a pattern of hair growth more like the human, and has been used to study wound healing (Winter, 1972). The application of corticosteroids can induce atrophy in pig skin and it has been shown that different compounds cause different degrees of epidermal thinning. We have also shown that there is a dose response effect (Winter & Wilson, 1975).

We now report a further study using this animal model to compare the effect of a new glucocorticoid ointment, clobetasone butyrate, with five other topically active corticosteroid ointments.

METHOD

Two young female Large White pigs from a pedigree herd were used. They were 4 months old at the beginning of the study and each weighed approximately 30 kg. The pigs were acclimatized in the animal house for 2 weeks. Their skin was clipped, washed and shaved, rinsed and dried. A biopsy specimen of untreated skin was taken. Areas of skin, measuring approximately 16 × 2·5 cm, on either side of the midline on the back of each pig, were delineated with strips of porous elastic plaster. The preparations to be tested were packed in coded identical 5 g tubes, the key to the code being held by an independent observer until the experiment was completed. Each marked strip on the first pig had one of the coded preparations assigned to it. The procedure was duplicated in the second animal but the preparations were allocated differently, so that each ointment was tested on the two animals at different anatomical sites.

The contents of one 5 g tube were applied evenly to the appropriate strip of skin. The applications were made daily 5 days a week for 7 weeks. The strips were covered with natural grade polythene film held in place with plaster. Biopsy specimens from each strip were taken at 1, 3, 5 and 7 weeks and the wounds closed with silk sutures. Care was taken to select areas remote from previous biopsy sites.

The skin specimens were fixed in formol saline and embedded in wax. From each specimen 30 serial sections 10 μm thick were prepared. Twenty sections in series were stained with haematoxylin and eosin, five were stained by the Weigert and van Geison method and five with Masson's trichrome stain.

The serial sections were examined under the microscope and the different preparations ranked subjectively according to their atrophogenic effect. A quantitative assessment was also made by projecting five representative sections of skin from each treatment site at each time interval in each pig at × 150 magnification and tracing the outline of a standard length of epidermis on paper. The cross-sectional areas were obtained by planimetry, and the average thickness of the epidermis was calculated from these data. All assessments were made and reported upon 'blind': the code was broken only on completion of the experiment.

The following seven ointments were used: fluocinolone acetonide 0·025%, hydrocortisone 1%, flurandrenolone 0·05% and 0·0125%, triamcinolone acetonide 0·1%, clobetasone butyrate 0·05% and an unmedicated base. All the corticosteroids used were in their marketed formulations; the unmedicated base was that used in the clobetasone butyrate preparation.

RESULTS

When the sections were examined under the microscope some specimens showed pronounced thinning of the epidermis with loss of the granular layer and flattening of the epidermal–dermal border. The results indicated that each ointment could be assigned to one of three categories according to the degree of its atrophogenic effect—marked, moderate to slight, or no effect at all (Table 1). The structure of the epidermis in the skin treated for 3 weeks with unmedicated base is illustrated in Fig. 1a. That treated with 0·05% clobetasone butyrate (Fig. 1b) shows by comparison some loss of the granular layer but otherwise little difference. The photographs of epidermis from skin treated with 0·1%

TABLE 1. The comparative atrophic effect of 7 weeks' application, under polythene occlusion, of various ointments on the skin of the domestic pig, as judged by histological appearance

Ointment	Concentration (%)	Atrophogenic ranking
Flurandrenolone	0·05	Marked effect
Fluocinolone acetonide	0·025	
Triamcinolone acetonide	0·1	Moderate to slight effect
Flurandrenolone	0·0125	
Clobetasone butyrate	0·05	
Unmedicated base	1	No effect
Hydrocortisone	1	

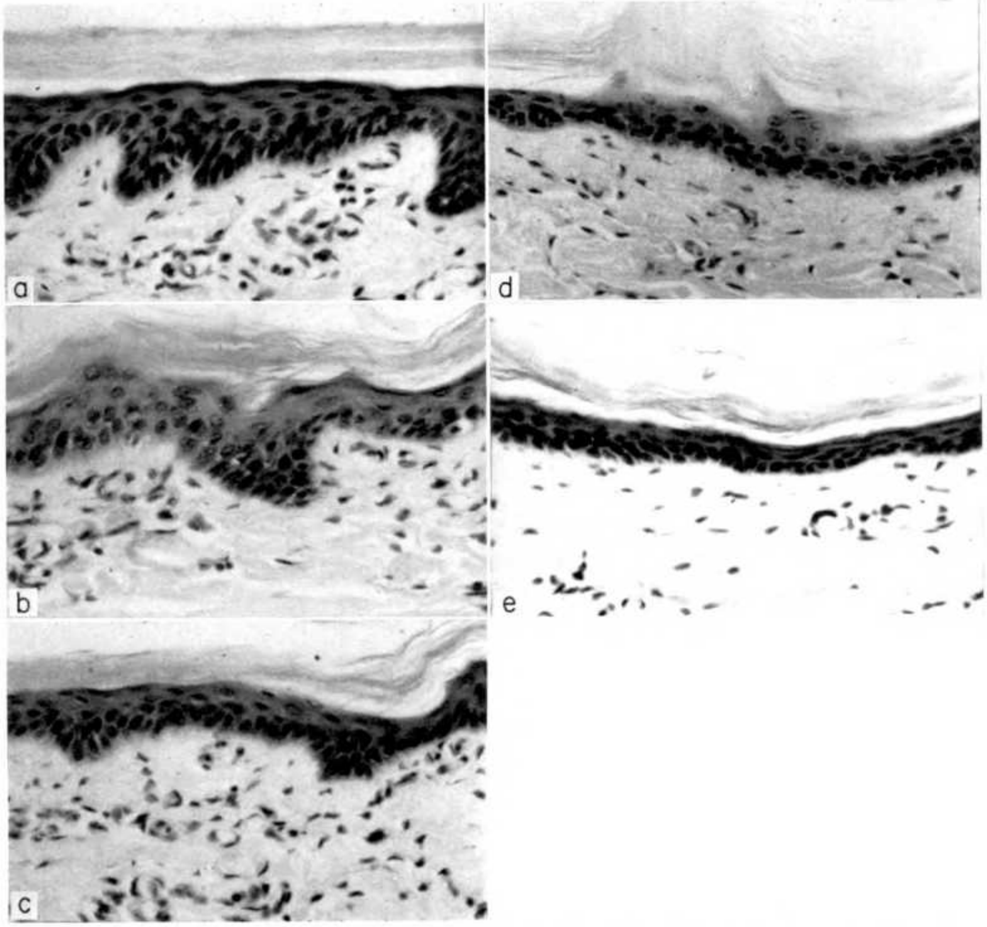

FIGURE 1. Epidermis of pig skin treated with (a) unmedicated base; (b) 0·05% clobetasone butyrate; (c) 0·1% triamcinolone; (d) 0·05% flurandrenolone; (e) 0·025% fluocinolone acetonide; for 3 weeks under polythene occlusion (H & E, ×450).

triamcinolone acetonide (Fig. 1c), 0·05% flurandrenolone (Fig. 1d) and 0·025% fluocinolone acetonide (Fig. 1e) illustrate total disappearance of the granular layer, progressive thinning of the epidermis as a whole and flattening of the epidermal–dermal border. Flurandrenolone at a concentration of 0·0125% had a more marked atrophogenic effect than clobetasone butyrate but a lesser effect than 0·1% triamcinolone acetonide.

The results of the quantitative assessment are shown in Table 2. When the experiment began the mean thickness of the epidermis in untreated skin was 65 μm. At the end of the experiment the thinnest epidermis was at the site treated with fluocinolone acetonide (41 μm) and the least affected was that treated with hydrocortisone (63 μm). In the main these measurements support the ranking order (Table 1) established qualitatively.

DISCUSSION

The results of experiments using the pig model have been consistent. Ointment or cream formulations of fluocinolone acetonide 0·025% and hydrocortisone 1%, together with the appropriate unmedicated

TABLE 2. The effect on epidermal thickness of various ointments applied under polythene occlusion to pig skin

Treatment	Mean projected area ± s.e. mean (cm²)	Thickness of epidermis (μm)	Differential thickness treated-normal skin (μm)
Untreated skin	30 ± 0·8	65	—
Fluocinolone acetonide 0·025%	19 ± 1·0	41	−24
Flurandrenolone 0·05%	21 ± 0·8	45	−20
Triamcinolone acetonide 0·1%	25 ± 1·2	53	−12
Flurandrenolone 0·0125%	26 ± 1·4	55	−10
Clobetasone butyrate 0·05%	27 ± 1·1	57	−8
Unmedicated base	27 ± 1·2	57	−8
Hydrocortisone 1·0%	29 ± 1·1	63	−2

Specimens of skin obtained at biopsy after 1, 3, 5 and 7 weeks. Five different histological sections at each time interval were projected × 150 magnification and the area of epidermal tissue measured by planimetry.

base, have been included in each of a number of experiments. On all occasions fluocinolone acetonide has been observed to have a marked thinning effect, and both hydrocortisone and unmedicated base to have little or no effect. Other topically active steroid formulations which have been tested on more than one occasion have also given consistent results. However, the precision of the method is such that too great a reliance can not be placed upon the order in which the steroids are ranked within each of the three broad categories of marked, moderate to slight, or no effect.

Fluocinolone acetonide has been included as a reference compound in an ettempt to reveal any

TABLE 3. A comparison of the ranking orders of effect of various steroids in inducing atrophy of pig skin (applied as ointments) and vasoconstriction of human skin (applied as alcoholic solutions, fluocinolone acetonide = 100 being used as a standard)

Atrophogenic test	%	Ranking* order	Vasoconstriction test	%	Ranking* order
Flurandrenolone	0·05	1	Clobetasone butyrate	263	1
Fluocinolone acetonide	0·025	2	Fluocinolone acetonide	100	2
Triamcinolone acetonide	0·1	3	Triamcinolone acetonide	75	3
Flurandrenolone	0·0125	4	Flurandrenolone	46	4
Clobetasone butyrate	0·05	5	Hydrocortisone	< 0·1	5
Hydrocortisone	1	6			

* In order of decreasing activity.

correlation that might exist between the vasoconstriction activity of a corticosteroid and its skin thinning effect.

In Table 3 the compounds tested in this study are ranked in descending order according to the effect seen in pig skin and compared with their ranking in the vasoconstrictor test described by McKenzie & Atkinson (1964). Their method of measuring vasoconstriction employs alcoholic solutions of the test compounds whereas for the atrophy study we used the marketed formulations, at a variety of concentrations and in differing ointment bases. Thus the dose response effect observable in clinical topical activity (Munro & Wilson, 1975) and the influence of formulation on penetration (Sarkany *et al.*, 1965) must be taken into account when comparing the data in Table 3. Nevertheless, certain inconsistencies can be seen. Flurandrenolone, ranking lower than triamcinolone acetonide in the vasoconstrictor test, caused a greater degree of skin thinning when applied at half the concentration. More strikingly, the 0·05% clobetasone butyrate ointment caused considerably less epidermal atrophy than flurandrenolone ointment in the same concentration, and this is at variance with the vasoconstrictor values of these two steroids, the rankings being inversely correlated.

Berliner *et al.* (1970) have ranked a number of glucocorticoids according to the degree of inhibition caused by each to the growth of fibroblasts in tissue culture. This assay has been claimed to predict intrinsic topical anti-inflammatory activity. This is unlikely, for the order of activity assigned to the three most widely used topical corticosteroids —fluocinolone acetonide > triamcinolone acetonide > betamethasone valerate—does not accord with clinical experience (Williams *et al.*, 1964). The effect of a glucocorticoid on fibroblasts, it might be thought, is more likely to indicate a steroid's propensity to cause connective tissue changes than its anti-inflammatory activity. However, our results do not support this. We found little difference between fluocinolone acetonide 0·025% and betamethasone valerate 0·1% (Winter & Wilson, 1975) but a lesser effect with triamcinolone 0·1%. Thus the fibroblast assay correlates neither with the vasoconstriction assay, nor with the effect of formulated steroids on the epidermis of the pig, nor with clinical topical activity.

By the time atrophy of the skin becomes obvious in patients using topical steroids they have been treated long enough for the dermis as well as the epidermis to be affected. To treat animals for longer than 2 months is impracticable, and after 7 weeks (the time of our last observations), the changes seen were still largely confined to the epidermis. Similar epidermal changes, after a similar interval, are seen in human skin (Winter & Wilson, 1975). If, as seems likely, epidermal thinning precedes dermal thinning then a preparation which has a lesser effect on the epidermis will have a lesser, or delayed, effect on the dermis.

Clinically observable atrophy of the skin can be a consequence of corticosteroid treatment whether administered locally or systemically. Although we were unable to detect any thinning of pig skin after the application of hydrocortisone this adrenocorticoid is not devoid of the potential to cause atrophy, for patients with Cushing's syndrome have diffuse thinning of the skin and striae. There is no evidence that the presence in the molecule of a halogenated atom, *per se*, makes a glucocorticoid more likely to be atrophogenic. Prednisolone causes atrophy when given orally and two other non-halogenated topical steroids, the commercially available des flurotriamcinolone acetonide (desonide) and an experimental compound, have both been shown to cause marked thinning in pig skin (Winter & Wilson, 1975).

Atrophy resulting from topical corticosteroids can probably be avoided if they are used correctly. However there would be advantage in having a compound in which there was a separation of local anti-inflammatory from atrophogenic activity. Given that the model described has some relevance to human clinical atrophy, clobetasone butyrate appears to have such a dissociation. Formulated as an ointment and a cream, clobetasone butyrate has been shown to have good clinical activity in short-term comparative trials and minimal effect on hypothalamic–pituitary–adrenal function (Munro & Wilson, 1975). However, only in the long term, under the conditions of everyday clinical use, can this observation be validated.

REFERENCES

BERLINER, D.L., BARTLEY, M.H., KENNER, G.H. & JOE, W.S.S. (1970) Activity of anti-inflammatory steroids upon fibroblasts and bones. *British Journal of Dermatology*, **82**, Supplement 6, 53.

McKENZIE, A.W. & ATKINSON, R.M. (1964) Topical activities of betamethasone esters in man. *Archives of Dermatology*, **89**, 741.

MUNRO, D.D. & WILSON, L. (1975) Clobetasone butyrate, a new topical corticosteroid: its clinical activity and effects on pituitary adrenal axis function and a model of epidermal atrophy. *British Medical Journal*, **iii**, 626.

SARKANY, I., HADGRAFT, J.W., CARON, G.A. & BARNETT, C.W. (1965) The role of vehicles in the percutaneous absorption of corticosteroids. *British Journal of Dermatology*, **77**, 569.

WILLIAMS, D.I., WILKINSON, D.S., OVERTON, J., McKENNA, W.B., LYELL, A. & CHURCH, R. (1964) Betamethasone 17-valerate: a new topical corticosteroid. *Lancet*, **i**, 1177.

WINTER, G.D. (1972) In: *Epidermal Wound Healing* (Ed. by H.I. Maibach and D.T. Ronee), p. 71. Year Book Medical Publishers Inc., Chicago.

WINTER, G.D. & WILSON, L. (1975) Corticosteroid induced atrophy in the skin of the domestic pig. In: *Mechanisms of Topical Corticosteroid Activity* (Ed. by L. Wilson and R. Marks), in press. Churchill Livingstone, Edinburgh and London.

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Ultrasonic methods for sampling human skin micro-organisms

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SUMMARY

Methods of sampling the microflora of human skin by means of a generator of ultrasonic impulses were compared with the standard washing method in which the skin surface is rubbed manually. The ultrasonic method, at a power setting of 50 W or more, gave essentially similar results to those obtained by the standard method but removed fewer corneocytes.

Three types of method have been used in quantitative studies of the microflora of human skin; impression methods, washing methods and biopsy methods (Noble & Somerville, 1974). Impression methods estimate the number of microcolonies on the area of skin sampled while washing methods aim to disperse the microcolonies and enumerate the viable cells. The most widely used washing method is that described by Williamson & Kligman (1965) in which a non-ionic detergent wash fluid is placed in a cylinder held to the skin and the skin surface is rubbed firmly with a blunt rod for 2 min. This method gives reproducible results but is time consuming, and the force and speed of the rubbing procedure are difficult to standardize. When the skin is damaged the procedure becomes quite painful.

Ultrasonic generators have been developed in recent years for a variety of cleaning and disintegrating tasks. A metal probe is set in vibration electrically and impulses travel from the face of the probe into an aqueous medium. The repeated compressions and rarefactions are believed to cause cavitation and the production of tiny bubbles which dislodge, disperse and, at a sufficiently high power density, disrupt material suspended in the medium. At high powers even bacterial cells can be disrupted, but at low powers the main application is in cleaning. It seemed likely that ultrasonic impulses could dislodge the organisms from human skin and disperse them into a detergent fluid without harming the subject and without killing the organisms. Such a procedure might be more reproducible and possibly less painful than the standard wash method of Williamson & Kligman. Moreover, the use of ultra-sound might make sampling of areas of skin bearing sebaceous follicles more informative, particularly in the study of acne vulgaris. We endeavoured to assess the usefulness of an ultrasonic method in sampling the microflora of human skin and the power and exposure time required. We compared the ultrasonic method with the standard washing method of Williamson & Kligman.

MATERIALS AND METHODS

Nine volunteers participated in these studies. All were healthy adult laboratory workers. Except where other sites are mentioned, all samples were taken from the volar forearm.

Standard washing method. A sterile stainless-steel cylinder (26·5 mm internal diameter) was held to the skin and 1 ml of 0·1% Triton X-100 in 0·075 M phosphate buffer, pH 7·9 (Williamson & Kligman, 1965) was added. The skin was rubbed with a blunt glass scrubber for 1 min and the fluid was then removed. The procedure was repeated without moving the cylinder and the two washes were pooled and mixed. In the serial scrub experiment, the scrubbing time was reduced to 30 s and the five successive scrubs of the same site were processed separately.

Ultrasonic sampling method. Ultrasonic impulses were generated by a pretuned Rapidis-300 generator fitted with a probe 25 mm in diameter and with a power regulator (Ultrasonics Ltd., Shipley, Yorks.). A steel cylinder similar to that used for the manual washing procedure was held to the skin surface and 2 ml of buffered Triton X-100 was added. The probe was lowered into the cylinder until the face was just immersed in the fluid, yet did not touch the skin and the power switched on for a timed interval. The power setting and the time of exposure to ultrasonic impulses were varied in the different experiments. The probe was disinfected with alcohol-soaked gauze initially and between experiments on different volunteers.

Bacteriological methods. Serial 10-fold dilutions of each sample were prepared in wash fluid diluted 1 in 2 with distilled water, and single drops (0·0234 ml) were placed on nutrient agar and on nutrient agar containing 1% 'Tween 80'. In addition 0·2 ml was spread on the surface of a plate of the latter medium. The plates were incubated at 30°C for 48 h and colony counts were then made.

Cytological methods. Corneocyte counts (McGinley, Marples & Plewig, 1969) were performed on the remainder of the undiluted samples. A 1 in 2 dilution of the sample was made by mixing 12 drops of sample, 11 drop of distilled water and 1 drop of membrane-filtered 2% crystal violet solution as prepared for the Gram stain. The epithelial cells were counted in a Fuchs-Rosenthal haemocytometer.

RESULTS

Effect of varying the power and duration of ultrasonic treatment. A low power setting of 30 W was compared to bilateral standard washes in three volunteers. Four sites, two on each forearm, were sampled by the ultrasonic method for 15, 30 45 and 60s respectively. The colony counts and corneocyte counts after treatment of varying duration were not significantly different and the results were therefore pooled. The geometric mean counts of both organisms and corneocytes obtained by the ultrasonic method were significantly lower than those obtained by the standard wash method (Table 1). No volunteer complained of pain and no signs of damage to the skin were seen on the sites exposed to the ultrasonic impulses.

A power setting of 90 W was then investigated as before in eight subjects and at a time of 60 s only in three subjects. As before, the times of exposure to the ultrasonic impulses did not differ significantly and were pooled. The numbers of organisms removed by the ultrasonic method were not significantly different from those removed by the standard wash but significantly fewer corneocytes were removed by the ultrasonic method (Table 1).

At this power setting (90 W) several subjects complained of a pricking sensation from the ultrasonic

TABLE 1. Results of comparisons of the standard wash method with the ultrasonic method at powers of 30 W and 90 W

	Method		F ratio	*P*	Method		F ratio	*P*
	Standard	Ultrasonic 30 W			Standard	Ultrasonic 90 W		
Organisms	3 590	379	6·12	<0·05	2 645	3 999	2.34	NS
Subjects	—	—	14·72	<0·01	—	—	11·38	<0·01
Corneocytes	69 100	8 770	45·33	<0·01	98 500	49 050	16·18	<0·01
Subjects	—	—	<1	NS	—	—	9·97	NS
Total samples	6	12			22	35	—	—

treatment and in several of these sites, usually but not restricted to the sites exposed for the longest duration, a distinct wealing response occurred some minutes after the sampling procedure.

In both experiments variations between subjects were significant and a major source of variation for the organisms counts but not for the corneocyte counts. Even so a one-way analysis of variance on the combined results indicated that all three methods could be distinguished on corneocyte counts. Counts of the total number of organisms did not differ significantly between the 90 W samples and standard wash samples but both were significantly higher than the samples obtained at 30 W.

Effect of serial sampling. In five volunteers, five successive 30 s standard washes were taken from the same site on one arm and five successive 10 s ultrasonic samples with the power adjusted to 50 W were

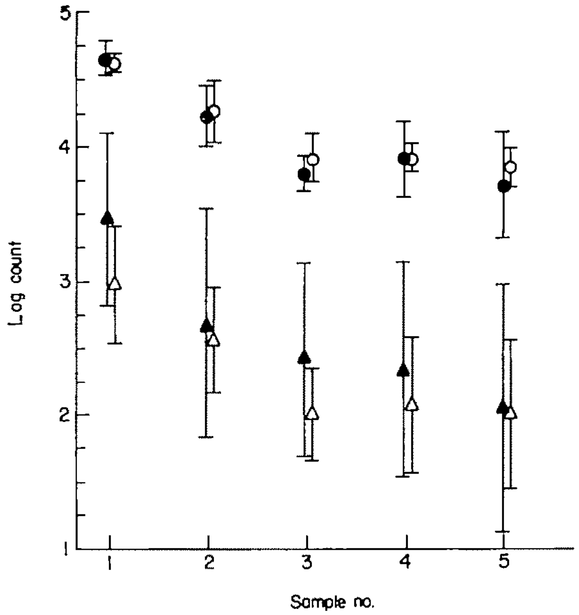

FIGURE 1. Counts of organisms (triangles) and corneocytes (circles) from successive samples taken by 30 s standard wash (black symbols) or 10 s ultrasound (white symbols). Bars indicate 95% confidence limits of the means.

TABLE 2. Effect of serial sampling from the same site

	Count obtained in sample no.				
	1	2	3	4	5
30 s Standard wash: no. of colonies	2930	489	271	220	111
10 s Ultrasound 50 W: no. of colonies	963	372	102	119	103
30 s Standard wash: corneocytes	44800	17000	6300	8050	5080
10 s Ultrasound 50 W: corneocytes	42900	18500	8120	8220	6840

taken from the symmetrical site on the opposite arm. There were no subjective complaints. The results are shown in Fig. 1 on a logarithmic scale and in Table 2 as the geometric mean counts.

The serial corneocyte counts were almost identical in the two series. The number of corneocytes removed was less in the second sample than the first and fell further in the third sample. The counts then appeared to level off at around 7000 corneocytes per sample. The non-linearity of the response could be confirmed statistically.

Taken together the results of colony counts by both methods indicate a progressive fall in the number of organisms in successive samples. Inspection of Fig. 1 shows that the amount of variation within samples was less with the ultrasound method than with the standard method in all the pairs of colony counts and in three of the five corneocyte count pairs. About half of the variation in the colony counts could be attributed to differences between subjects but subjects were not a significant source of variation in the corneocyte counts.

Ultra-sound on the head. An attempt was made to take serial ultrasonic samples from the forehead in one volunteer. Although this was technically feasible, the subjective sensation of bone-conducted sound, even for 10 s at 50 W, was only just endurable. Further volunteers were not sought.

DISCUSSION

The principal findings of this study were first, that an ultrasonic method for sampling the skin microflora in healthy adults was feasible and at power settings of higher than 30 W gave results that were essentially equivalent to those obtained by the standard detergent wash technique of Williamson & Kligman. A power setting of 90 W was excessive, in that damage to the skin and pain to the volunteers became evident. A power of 50 W is available in hand-held transducers which, with a modified probe, would be satisfactory in use. A sampling time of 10–15 s seemed to be as effective as a longer period of exposure to the ultrasonic impulses.

Secondly, the results of the corneocyte counts differed from those of the counts of micro-organisms. At 90 W, when ultrasonic treatment was definitely damaging the skin, significantly fewer epithelial cells were removed than were removed by the standard wash method. The numbers of organisms removed were, however, the same or a little higher in the ultrasound samples than in the standard wash samples. This suggests that both methods are equally efficient in removing organisms but that the standard wash method grinds more deeply than the ultrasonic method into the compact stratum corneum, which is free of micro-organisms. This confirms the extremely superficial location of the habitat in which the normal flora exists in areas bearing only vellus hairs (Kligman, 1965) and weighs against the occurrence of many organisms deep in the stratum corneum as is suggested by tape-stripping experiments (Ulrich, 1965; Noble & Somerville, 1974). In stripping methods the adhesive tape cannot adapt itself to the contours of the skin surface particularly at the infundibula of hair follicles, and organisms appear to persist even after twenty strippings.

Thirdly, the ways in which the number of organisms or the number of corneocytes became reduced on serial sampling of the same site did not differ between the two methods, even though the duration of the ultrasonic exposure was only 10 s at 50 W per sample while the time of standard washing was 30 s per sample. Here, where the parameters of the methods were adjusted to give equivalent sampling, the lower variation in the results of the ultrasonic method indicates an improvement in reproducibility. The corneocyte counts in serial samples of the same sites appeared to approximate to a constant count after the first two samples suggesting that the desquamating layer had been passed and that cells were being removed from the compact stratum corneum. The low numbers of organisms and the greater variation in organisms than in corneocyte counts precluded any determination of a residual flora.

Finally, the use of ultrasonic methods on the head was found to be impractical. Bone-conducted high-pitched sound was felt to be very unpleasant, ruling out applications in the study of acne.

In these studies, the importance of allowing for variation between individual volunteers was again demonstrated. Particularly when the number of micro-organisms was being measured, was it necessary to try all treatments on each subject. Corneocyte counts were, however, very similar from person to person, suggesting that it is not the microgeography and micro-anatomy that determine the density of the microflora but other less well-understood factors, presumably including skin permeability, sweat rates and the amount of nutritious and deleterious compounds deposited on the skin from both endogenous and exogenous sources.

REFERENCES

KLIGMAN, A.M. (1965) The bacteriology of normal skin. In: *Skin Bacteria and their Role in Infection* (Ed. by H.I. Maibach and G. Hildick-Smith). McGraw Hill, New York.

MCGINLEY, K.J., MARPLES, R.R. & PLEWIG, G. (1969) A method for visualizing and quantitating the desquamating portion of the human stratum corneum. *Journal of Investigative Dermatology*, 53, 107.

NOBLE, W.C. & SOMERVILLE, D.A. (1974) *Microbiology of Human Skin.* W.B.Saunders, London.

ULRICH, J.A. (1965) Dynamics of bacterial skin populations. In: *Skin Bacteria and their Role in Infection* (Ed. by H.I.Maibach and G.Hildick-Smith). McGraw Hill, New York.

WILLIAMSON, P. & KLIGMAN, A.M. (1965) A new method for the quantitative investigation of cutaneous bacteria. *Journal of Investigative Dermatology*, 45, 498.

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Cell division and metabolic activity of naevus cells

THE RELATIONSHIPS BETWEEN ANATOMY AND BEHAVIOUR IN MOLES

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SUMMARY

Thirty moles have been studied by *in vitro* techniques employing tritiated precursor compounds in order to demonstrate replicative and metabolic activity in naevus cells. Autoradiographs prepared from the incubated tissue showed that replicative activity occurred at all anatomical levels within the naevi. Synthesis of macro-molecules also occurred throughout the mole, but more actively in the junctional and superficial zones. The term 'Junctional Activity' does not appear to have any meaning in cell kinetic terms, and is not associated with any proliferative capacity.

Moles are mysterious. Clinically they seem to have a definite life cycle in which they appear slowly to enlarge and eventually become less prominent and may even disappear. An important aspect of this evolutionary process in the individual mole has been accepted as being the proliferation of naevus cells in the 'junctional zone' (junctional activity) and the delivery of packets of naevus cells to the dermis—a process called by Unna (1893) 'Abtropfung'. The implication is that naevus cells divide—at least in some stage of their history. This is difficult to reconcile with the reports in the literature that assert that mitotic activity is rare in ordinary moles. Thus, Miescher (1927) commented that mitoses are exceptionally infrequent or absent in intra-epidermal cell nests. Masson (1951) stated 'The numerical increase of the nevic cells depends on their own slow proliferation, rarely mitotic, chiefly amitotic'. Lund & Stobbe (1949) found mitotic figures in only six out of thirty juvenile naevi—they were all in junctional or immediately subepidermal sites. Stegmaier & Montgomery (1953) studied moles from ninety-five children and found only one section with mitotic figures. Stegmaier (1959) considered that in later life junctional activity usually comes to a halt, proliferation ceases, and the naevus cells may be involved with fibrosis and fat deposition, eventually disappearing or falling off as skin tags.

A corollary of the 'life cycle' concept of the mole might also be that metabolic activity varies at different points in the evolution of naevus cells. Petzoldt (1967) using histochemical techniques demonstrated considerable enzyme activity for both glycolysis and Krebs cycle. The glycolytic activity was found to be much higher in the superficial than in the deep dermal naevus cells, and it was even higher in areas of 'junctional activity'. Krebs cycle activity was on the other hand reduced in these areas and the overall pattern did not suggest that the increased activity was due to an increased rate of mitosis or of melanin synthesis.

In this study, we have attempted to find out if the naevus cells divide, and if so, how rapidly, and at which levels the greatest replicative activity occurs. We have studied synthetic activity in naevus cells for nucleic acids and proteins comparing the degree of activity in different anatomical zones. Our overall intention has been to see if the presence of 'junctional activity' in a mole is related to greater synthetic and replicative activity and to see if the term 'junctional activity' had any meaning in cell kinetic terms.

METHODS

Thirty moles (11 compound and 19 intradermal) of differing clinical appearance and in subjects of all age groups were excised and cut into thin slices 1–2 mm thick using sharp disposable scalpels. Five of these were congenital moles, i.e. they were present at birth, were flattened or plaque like, were 'hairy' and had an irregular margin. Each slice was incubated in Eagle's minimum essential medium in the presence of 2·5 μCi/ml of one of the tritiated precursor compounds, thymidine, proline or cytidine. Incubation proceeded for 2½ h at 37°C in an atmosphere of 95% air and 5% CO_2. At the end of this period, the sections were fixed and processed autoradiographically using the dipping method of Joftes & Warren (1955).

In the sections incubated with tritiated thymidine, the labelling index (LI) of the epidermal cells was estimated by conventional means, as the percentage of labelled basal and suprabasal cells of the total number of basal cells. Similarly the LI for the naevus cells was estimated as the percentage of heavily labelled naevus cells of the total number of naevus cells in the section. In some of the sections, areas near the centre showed a lesser degree of labelling—the result presumably of the difficulty that thymidine has in diffusing through tissues. In these circumstances, only areas showing uniform and adequate labelling were evaluated.

When examining sections derived from slices incubated with tritiated proline, areas evaluated were equidistant from the cut edges in order to ensure a comparable exposure to the diffusing precursor. Similar precautions were taken when estimating tritiated cytidine incorporation. Especial care was necessary with tissue incubated with this precursor, as autoradiographs showed a greater variability in labelling.

RESULTS

(a) *Thymidine incorporation*

Thymidine incorporation in naevus cells was noted in varying amounts in all the specimens. Labelled cells occurred at all levels of the mole, there being no preference for any particular anatomical zone. Fig. 1 (a and b) shows labelling in subepidermal and upper dermal zones. It was sometimes difficult to be completely certain that a labelled cell was in fact a naevus cell. However, in most cases, the morphology of the labelled cell, the nature of the adjacent cells and the relationship of the labelled cell to them enabled the assumption to be made with confidence.

The overall labelling index of the epidermal cells was 6·1 ± 2·2% and the overall LI of the naevus cells was $6{\cdot}0 \pm 4{\cdot}1 \times 10^{-2}$. Fig. 2 shows the number of moles examined with the degrees of labelling found in them. It will be noted that most naevi have an LI between 0·01 and 0·1%. The five congenital moles had labelling indices of 4·0, 5·0, 1·0, 1·3, 25·0 (mean 7·3) $\times 10^{-2}$.

(b) *Proline incorporation*

Proline incorporation occurred in all the naevi, and was measured as the mean number of autoradiographic grains per cell type. There was a gradation in the amount of incorporation with a higher degree of labelling in the superficial than in the deep dermal zones. Fig. 3 (a and b) shows heavy labelling of upper dermal naevus cells, comparable to that shown by epidermal basal cells. Fig. 4 demonstrates that proline uptake in the junctional zone is of the same order as that in the basal cells of the epidermis, and is about five times greater than that in the deep dermal naevus cells.

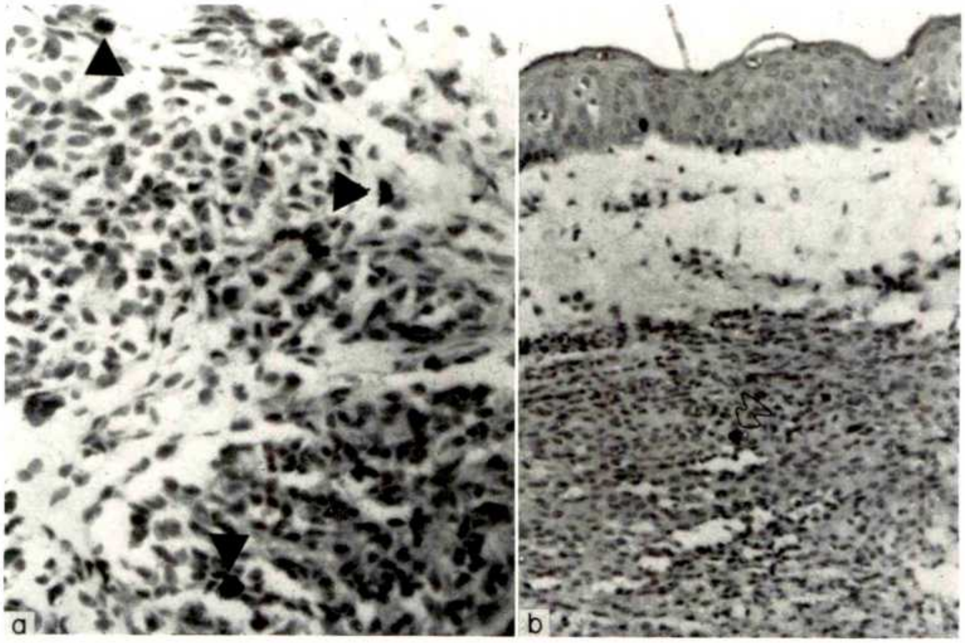

FIGURE 1. Autoradiographs from naevi that have been incubated in the presence of tritiated thymidine to show the presence of labelled naevus cells in (a) the papillary zone (H & E, × 150), and (b) the upper dermis (H & E, × 90). Arrows indicate labelled cells.

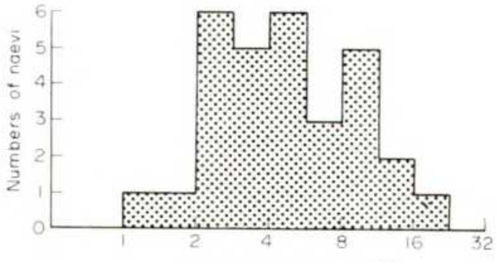

FIGURE 2. Histogram showing the number of naevi with different labelling indices.

(c) *Cytidine incorporation*

Cytidine incorporation was more difficult to interpret. Fig. 5 shows well that there were some adjacent

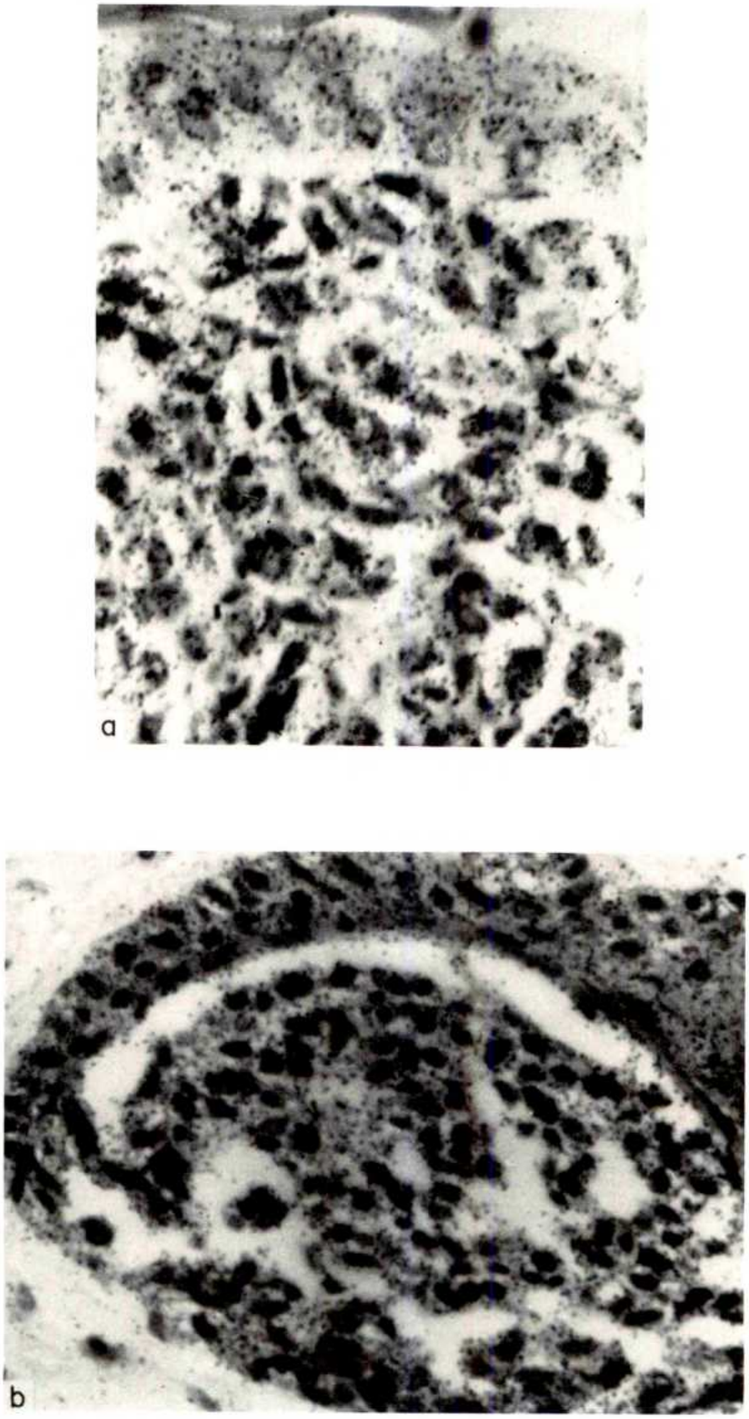

FIGURE 3 (a and b). These autoradiographs show labelling due to tritiated proline. There are similar levels of incorporation into epidermal basal cells and subjacent naevus cells (H & E, × 150).

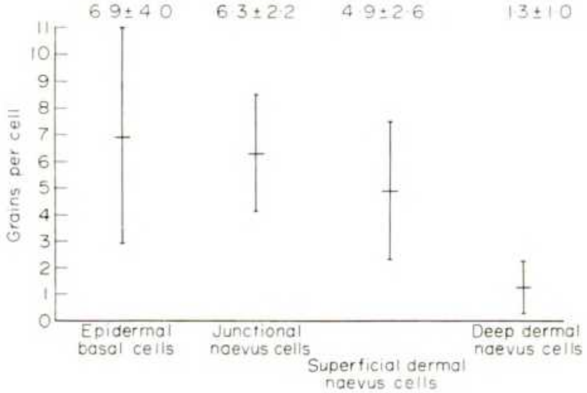

FIGURE 4. Diagram showing range of proline incorporation into cells at various anatomical levels in moles. Uptake in epidermal basal cells and in junctional naevus cells is much higher than that in the deeper naevus cells.

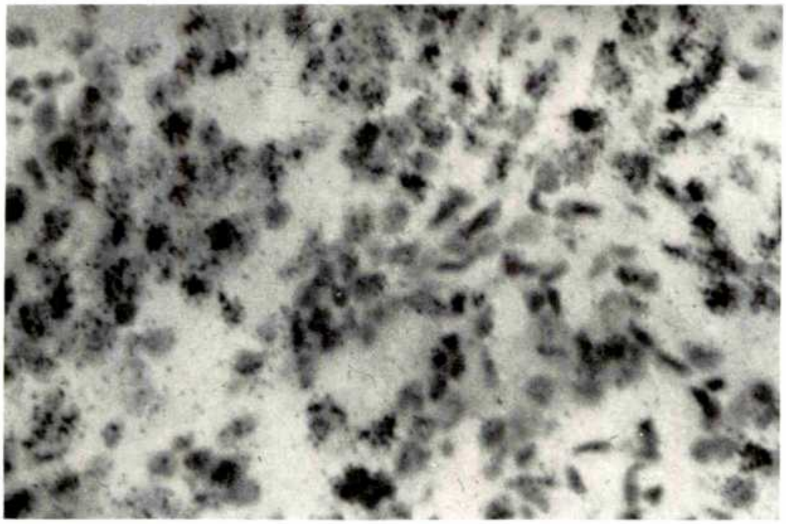

FIGURE 5. This autoradiograph shows labelling due to tritiated cytidine. Incorporation in adjacent naevus cells varies considerably (H & E, × 150).

areas with different degrees of incorporation, and this variation cannot be explained by differences in exposure to the diffusing cytidine. However, there did appear to be a greater incorporation in the junctional and superficial zones than in the deeper parts of the dermis. Table 1 shows that when comparing compound naevi with junctional activity and intradermal naevi with minimal junctional activity, there was no difference in the thymidine and proline incorporation in the two groups.

COMMENT

Thymidine incorporation into naevus cells can be taken as evidence that DNA is being synthesized in these cells, and that they are, therefore, about to divide. The naevus cell LI varied between 0·1 and

TABLE 1. This table demonstrates that there is no significant difference in uptake of tritiated thymidine or proline in compound and intradermal naevi

	Compound naevi	Intradermal naevi
Thymidine		
Epidermal LI	6·6 ± 2·1	6·0 ± 2·2
Naevus cell LI	$6{\cdot}3 \pm 4{\cdot}0 \times 10^{-2}$	$5{\cdot}0 \pm 2{\cdot}8 \times 10^{-2}$
Proline		
Grains/cell		
Junctional	6·6 ± 2·8	7·1 ± 4·6
Superficial dermal	4·6 ± 1·7	5·0 ± 3·0
Deep dermal	1·2 ± 1·0	1·4 ± 1·0

0·01 which indicates that they are dividing at a rate of 1/100th that of the epidermal cells. Cells from all levels of the dermis and from the junctional nests were seen to be incorporating thymidine, and it was not possible to say that any one level was more active than any other. The cells in the junctional region appeared to be replicating no more actively than those in the deep dermis. Also the LI in the compound naevi was no higher than that in the intradermal naevi, and this again suggests that 'junctional activity' is not associated with increased mitotic proliferation of naevus cells. It is difficult to correlate these findings with other workers' observations that mitotic figures are rarely if ever seen in naevus cells. It might have been informative to correlate the degree and site of labelling with the stage in 'life history' of the mole. However, most donors of the moles in this series could not state with certainty how long their pigmented lesion had been present. In the five with 'congenital moles' only a small and non-significant difference in naevus cell labelling index could be detected. The mitotic phase in the germinative cell cycle is much shorter than the synthetic phase, and thymidine uptake is therefore a simpler (and more accurate) method of assessing replication than counting mitotic figures. Proline incorporation into cells is a function of protein synthesis. In this investigation we are comparing the amount of incorporation in different areas of the same tissue and therefore the size of the proline pool can be considered to be constant. It can be seen that all levels of naevi are synthetically active, the superficial naevus cells being more active than the deep dermal ones, and the cells of the junctional nests being more active than both. These findings correlate well with the histochemical observations of Petzoldt (1967), Paul & Illig (1974) and our own unpublished observations, that naevus cells in the junctional and upper dermal regions are metabolically more active than the deeper cells as judged by the activity of glycolytic and respiratory chain enzymes.

On the basis of this investigation, it would seem that junctional naevus cells have no special kinetic properties. 'Junctional activity' may well be one morphological pattern of naevus—a stage in its orderly progression—and its presence does not necessarily signify a potentiality for growth. In view of the absence of any undue replicative activity in the junctional zone, the relationship between 'junctional activity' and malignancy at this zone requires further elucidation. The enhanced respiratory enzyme activity (as evidenced by enzyme histochemical findings) and synthetic activity (shown by our present findings) of naevus cells in the upper dermis probably reflect the decreasing metabolism of these cells as they draw towards the end of their life span in the lower dermis. We find it interesting to note that they retain their replicative activity while in this phase vitality decreases. The genesis of mole cells is obscure but we believe that this study goes some way towards understanding their behaviour and we hope that further studies along these lines may throw light on the kind of biological niche occupied by naevus cells.

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REFERENCES

JOFTES, D.L. & WARREN, S. (1955) Simplified liquid emulsion autoradiography. *Journal of the Biological Photographic Association*, **23**, 145.

LUND, H.Z. & STOBBE, G.D. (1949) The natural history of the pigmented naevus. Factors of age and anatomic location. *American Journal of Pathology*, **24**, 1117.

MASSON, P. (1951) My conception of cellular naevi. *Cancer*, **4**, 9.

MIESCHER, G. (1927) Die Entstehung der boesartigen Melanome der Haut. *Virchows Archiv für Pathologische Anatomie und Physiologie und für Klinische Medizin*, **264**, 86.

PAUL, E. & ILLIG, L. (1974) Histochemische Untersuchung an dermalen Naevuszellnaevi. *Archiv für klinische und experimentelle Dermatologie*, **251**, 133.

PETZOLDT, D. (1967) Enzyme des energieliefernden Stoffwechsels in Naevuszellen. Eine histochemische Untersuchung. *Archiv für klinische und experimentelle Dermatologie*, **228**, 136.

STEGMAIER, O.C. (1959) Natural regression of the melanocytic naevus. *Journal of Investigative Dermatology*, **32**, 413.

STEGMAIER, O.C. & MONTGOMERY, G. (1953) Histopathologic studies of pigmented naevi in children. *Journal of Investigative Dermatology*, **20**, 51.

UNNA, P.G. (1893) Naevi und Naevocarcinome. *Berliner klinische Wochenschrift*, **30**, 14.

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Melanoma cytoplasmic humoral antibody test

A DIAGNOSTIC ADJUNCT

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SUMMARY

Detecting a high titre of antibody directed against the cytoplasm of malignant melanoma cells can help:

To diagnose pigmented skin lesions with awkward histopathological signs. The test is positive in about half of the early primary malignant melanomas and in all actively regressing halo naevi. It is negative in benign juvenile melanoma.

To prognosticate and provide a continuing assessment of the course of the disease in patients with known malignant melanoma. The test becomes negative when malignant melanoma metastasizes.

To alert patients in danger, such as those who have extensive pigmented naevi or have a family history of malignant melanoma, of the supervention of the disease.

MATERIALS AND METHODS

Sera were examined by the technique we have used previously (Copeman, *et al.*, 1973) for the demonstration of antibody to the cytoplasmic antigen of malignant melanoma cells. Smears of melanoma cells obtained from fresh biopsy specimens were snap frozen in a mixture of liquid nitrogen and isopentone at −160°C for 2 min and afterwards were air dried for 5 min. Then they were washed in freshly prepared phosphate buffered saline (PBS) (pH 7·3, 0·1 M) for 20 min. Every microscope slide had five smears, on to which in order was placed one of the following:

(a) A known positive serum from a patient with malignant melanoma. (b) A known negative serum from a patient with malignant melanoma. (c) Phosphate buffered saline (PBS) to test for purity of conjugate. (d) Normal human serum. (e) Serum under test.

All sera were diluted 1:4 in PBS. Slides were left for 30 min in a moist chamber, rinsed and then washed in PBS to remove unreacted sera. The smears were treated with 1 drop of goat anti-human gamma globulin labelled with fluorescein isothiocyanate (F-P Molar ratio 3·9—Wellcome Reagents) at a dilution of 1:16 and incubated for 30 min in a moist chamber. After washing in freshly prepared PBS for 1 h, they were mounted in a mixture of equal parts of PBS and glycerol.

The smears were examined by incident ultra-violet (epi) illumination using a Leitz Orthoplan microscope equipped with a HBO 200 mercury vapour light source. Ground glass Schott UGI, BG12 and KP 490 primary filters were used in the light path with a K530/513 secondary filter and a 45° inclined dichroic mirror set at position 3.

Positive results were recorded only when 25% or more of the total number of cells had their cytoplasm stained. The endpoint of the titration was the final maximum dilution that gave this requirement.

All positive sera were retested against malignant melanoma smears and also against batches of normal cells and other tumour cells. The normal cells were skin fibroblasts, liver, spleen, and lung; the tumour cells were neuroblastoma, carcinoma of stomach and of colon, osteosarcoma, and Wilms' nephroblastoma.

We receive as a routine the serum of each patient who has a pigmented skin lesion removed by a surgeon or a dermatologist in our Tumour Biology Group, at Westminster Hospital.

RESULTS

We tested 800 specimens of serum taken from patients with pigmented lesions on the skin. We are presenting the results according to their clinical and histopathological diagnoses.

Various pigmented and partially pigmented apparently benign skin lesions. All 263 were negative. The only positive test caused us to re-examine the histopathological appearance. The lesion then was diagnosed as malignant melanoma having been incorrectly named a benign juvenile melanoma.

Halo naevi. Of a new series of twenty-eight actively resolving naevi, twenty-seven were positive. The negative serum came from a patient with a mole appearing clinically to be active, but histopathological examination showed it to have resolved totally. The antibody titres are presented in Table 2.

Malignant melanoma of the skin. All tumours were positively identified by histopathological investigations. The total number of sera tested was 512. The results are presented in Tables 1 and 2. They show that one third of patients with early malignant melanoma of the skin have a significant antibody titre. When the tumour penetrates the dermis more deeply the test becomes negative.

Antibody specificity tests

Each positive serum has been tested against:

Normal tissues. Skin fibroblasts, liver, spleen and lung.

TABLE 1. Malignant melanoma of the skin: sera tested for antibody directed against the cytoplasm of malignant melanoma cells

Stage	No. of patients tested	% of total	No. positive	% positive
I	57	11	25	46
IIa	102	25	21	23
IIb	210	41	0	—
III	143	28	0	—

TABLE 2. Maximum titre of antibody serum that gave the staining requirements

No. of patients	% of total	Titre	Stage
Malignant melanoma			
1	2·5	1:64	I IIa
1	2·5	1:16	I
2	4	1:8	I
42	91	1:4	I IIa
Halo naevus			
5	18·5	1:16	All active
20	74	1:8	and
2	7·5	1:4	resolving

Cancer cells. Neuroblastoma, cancers of the stomach and colon, osteosarcoma and Wilms' tumour (nephroblastoma).

All tests against these substrate tissues were negative.

COMMENT

We believe our data demonstrate a useful additional diagnostic test to screen patients with, or susceptible to, malignant melanoma. The test detects humoral antibody directed against the cytoplasm of the living malignant melanoma cell and not against its plasma membrane. The biological significance of these antibodies, whether a chance concomitant, an epiphenomenon, and their relationship to cell-mediated immunological responses, does not concern us. Nor do we wish to discuss the reason for their disappearance from the serum—others have suggested a number of explanations among them the development of antiidiotypic antibodies.

Many histopathologists accept that the depth of penetration into the dermis of the cells of malignant melanomas (omitting the superficial Hutchinson–Debreuilh variety, and such matters as sex or race of the patient, and anatomical site) is of paramount importance in assessing prognosis. Nevertheless, even if judged on many serial sections and samples, this depth-rating may be imprecise. One third of the sera we have tested were positive in the more superficial (Stage I and IIa) malignant melanomas. In those patients whose tumours seem to have penetrated deeper, the test always has been negative. This negative finding, thus, is highly important because it indicates a grave prognosis. Moreover, if the serum, positive because of a primary malignant melanoma, becomes negative when tested on a later occasion, this may indicate that the tumour now has metastasized. Secondary deposits in such cases may remain occult clinically perhaps for some time. The test therefore could provide the first indication of an extension of the disease.

The halo naevus seems to be the only exception to this otherwise tumour-specific test. When this curious self-healing pigmented mole is in the process of resolving, a vigorous antibody response is noted and cytoplasmic malignant melanoma antibodies are found almost always. But on completion of this active process of resolution (a stage that only histopathological examination can reveal), or after total excision of the mole, the antibodies within a few weeks can be detected no longer. Furthermore, the sera from our patients with halo naevi did not react with other tumour antigens and substrate tissues so the test would seem to be very specific for actively regressing halo naevus and for early stages of malignant melanoma.

The serum of only one of the 264 patients with histopathologically diagnosed benign lesions was positive. Reassessment of the microscopical appearances revealed this tumour's malignancy. Thus in this case, at least, the test proved useful in diagnosis.

Patients known to have had a primary malignant melanoma may be followed up until the test becomes negative, a situation that can indicate the development of metastatic deposits or the deeper penetration by the cells of the primary melanoma. High risk patients—those with a vast number of moles, a congenital giant pigmented mole, or the history of familial malignant melanoma—could be regularly screened by this convenient serological test.

We consider that all patients with pigmented skin lesions in whom suspicion of malignant melanoma has been raised, in addition to having their tumour examined histopathologically, should have their serum examined for humoral cytoplasmic malignant melanoma antibodies. We wish to recommend, therefore, not an immunological exercise but a diagnostic adjunct and a method of clinical surveillance against malignant melanoma.

REFERENCE

COPEMAN, P.W.M., LEWIS, M.G., PHILLIPS, T.M. & ELLIOTT, P.G. (1973) Immunological associations of the halo naevus with malignant melanoma. *British Journal of Dermatology*, 88, 127.

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Case Reports

Primary amyloidosis and myelomatosis associated with excessive fibrinolytic activity

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SUMMARY

A case of primary amyloidosis is described in association with Bence-Jones myeloma and increased fibrinolytic activity. Epsilon aminocaproic acid reversed the fibrinolytic activity and clinical improvement was maintained with melphalan and prednisolone.

The association of primary amyloidosis with M-component paraprotein has been well recorded (Cathcart *et al.*, 1972; Osserman, 1965; Pick & Osserman, 1967). Primary, rather than secondary, amyloidosis tends to involve the skin and gut; in the presence of M-component paraprotein a peripheral neuropathy (Benson *et al.*, 1975) is frequently found. This may appear years before any other clinical features. Myocardial involvement with amyloid is a common finding (Dahlin, 1949) and is the cause of death in approximately half these patients (Josselson, 1950). The following patient showed all the above features in addition to a bleeding tendency caused by increased fibrinolytic activity.

CASE REPORT

In 1961, a 49-year-old married woman developed nocturnal paraesthesiae in both hands, for which she had a bilateral median nerve decompression in 1968 with some improvement. She developed extreme sensitivity to local pressure on her fingers and, in 1970, bruising and purpura of both hands, including the fingers, was first noticed. Further lesions developed around the neck, orbits and lips in 1971. Albuminuria, low serum albumin and a low level of serum IgA were noticed in 1972. Ankle oedema and paroxysmal nocturnal dyspnoea developed later in that year and, on two occasions, in September 1972 and March 1974, she developed bronchopneumonia.

When first seen at the Department of Dermatology at the General Infirmary at Leeds in May 1974 she was a nervous woman with extensive purpura around the orbits, on the eyelids, on the lips, around the neck and on pressure sites on the posterior and anterior axillary walls. The skin of the fingers was atrophic, shiny and purpuric. Shiny papules, about 1–2 mm in diameter, were present mainly on the dorsa of the fingers. Such was the sensitivity of her hands that a light touch produced an unpleasant stabbing sensation. The distribution of the hyperaesthesiae was not confined to any peripheral nerve distribution. All reflexes were sluggish, the ankle jerks being absent. She was in mild congestive cardiac failure. The tongue was normal.

Investigations. Haemoglobin 14·9 g%; WBC 8600/mm^3; platelets 238,000/mm^3; ESR 40 mm/h. The following haematological tests were normal: whole blood clotting time, bleeding time, prothrombin time, prothrombin consumption index, thrombin time, thromboplastin generation test, Factor V, Factor VIII, Factor XIII, serum fibrin degradation products, fibrinogen assay.

Intradermal injection of red cells produced no reaction. Euglobin lysis time 60–75 min on six occasions (Von Kaulla, 1963, as modified by Menon, 1967. Normal for age 120 min). Serum proteins 53 g/l (albumin 29 g/l, globulin 24 g/l); immunoglobulins, IgG 1100 mg%, IgA 98 mg%, IgM 47 mg%; immunoelectrophoresis against anti-lambda components—gross increase in free light chains; 24-h-urinary protein 3·78 g/l. Electrophoresis of urine showed gross increase in lambda light chains; urinary Bence-Jones protein positive; blood urea 20 mg%; creatinine clearance 62 ml/min. Sternal bone-marrow examination showed no abnormal plasma cells. Skeletal survey showed generalized widespread osteoporosis but there were no lytic lesions. ECG—low voltage with partial left bundle branch block. Skin biopsy from a finger showed amyloid deposits particularly around the blood vessels with a positive reaction to Congo red stain and thioflavin T fluorescence. Rectal biopsy showed massive infiltration of amyloid with identical staining characteristics. Nerve conduction studies showed absent sensory responses in both median nerves with small motor responses.

Treatment. Epsilon aminocaproic acid (Epsikapron) 7 g by mouth 12-hourly improved the bruising and paraesthesiae after 3 days and her euglobin lysis time rose to normal (120 min) within 5 days. After 3 weeks' therapy it was stopped and the euglobin lysis time fell back to 60 min. The purpura recurred in the next 2 weeks. Treatment with melphalan and prednisolone according to the schedule of Costa *et al.* (1973) was started. Melphalan was given as a loading dose of 9 mg (150 μg/kg/day) for 7 days with a maintenance dose of 3 mg (50 μg/kg/day). For the first 2 weeks prednisolone was given as 75 mg (1·25 mg/kg/day) followed by 50 mg (0·80 mg/kg/day) for 2 weeks, then 30 mg (0·50 mg/kg/day) for 2 weeks and, finally, 15 mg (0·25 mg/kg/day) for a month. The prednisolone was then stopped. The hyperaesthesiae became minimal and the purpura receded over the next 3 months. The euglobin lysis time was maintained at about 90 min. Quantitative estimation of the serum M-component showed a reduction to 430 mg% with the IgM fraction elevated to 177 mg%. Urinary 24-h-protein had come down to 0·9 g/litre and the free lambda light chains, although still present, were not so marked on urinary electrophoresis. Nerve conduction studies were, however, unchanged.

DISCUSSION

In this case there was direct evidence of skin and gut involvement by amyloid, and fibrinolytic activity in the blood was increased. Cardiac, peripheral nerve and renal involvement by amyloid also appeared likely.

The association of primary amyloidosis, myelomatosis and increased fibrinolytic activity has not been reported previously but abnormalities of fibrinolysis may not be uncommon in this group of diseases if looked for. Increased fibrinolytic activity was found in a case of myelomatosis (Sirridge, Bowman & Garber, 1958) and a case of primary amyloidosis (Redleaf *et al.*, 1963).

It seems likely that this patient's purpura may be produced in part by excessive plasminogen activator. Whatever the aetiology, epsilon aminocaproic acid reversed the fibrinolytic effect within 5 days and produced clinical improvement. This compound is a specific inhibitor of plasminogen activators which cause the conversion of inactive plasminogen to plasmin and its use in reversing the bleeding tendency produced by hyperplasminaemic states has been well documented (Redleaf *et al.*, 1963; Davidson *et al.*, 1969; Rowell, 1974).

Topical epsilon aminocaproic acid, used as compresses on the fingers for 20 min 3 times daily for 2 weeks, improved the local bruising and purpura as compared with a placebo. Topical epsilon aminocaproic acid has been used successfully (Kwaan & Silverman, 1973) in a case of hereditary haemorrhagic telangiectasia with increased plasminogen activator.

Oral epsilon aminocaproic acid was replaced by a combination of prednisolone and melphalan (Costa *et al.*, 1973) in order to treat the underlying myeloma. On this therapy her improvement has been maintained and 15 months later she is free from hyperaesthesia and has only mild purpura of the distal phalanges. The euglobin lysis time is only slightly decreased (95 min).

This case should draw attention to the advisability of investigating the fibrinolytic state, as well as other haematological parameters, in myeloma and amyloidosis. If this were done routinely, particularly in the patients presenting with purpura, increased fibrinolytic activity might be demonstrated more frequently.

ACKNOWLEDGMENTS

We are very grateful to Dr Bowyer for referring the patient and for allowing us to publish this report.

REFERENCES

Benson, M.D., Cohen, A.S., Brandt, K.D. & Cathcart, E.S. (1975) Neuropathy, M components and amyloid. *Lancet*, **i**, 10.

Cathcart, E.S., Ritchie, R.F., Cohen, A.S. & Brandt, K. (1972) Immunoglobulins and amyloidosis. *American Journal of Medicine*, **52**, 93.

Costa, G., Engle, L., Schilling, A., Carbone, P., Kochwa, S. & Glidewell, O. (1973) Melphalan and prednisolone: an effective combination for the treatment of multiple myeloma. *American Journal of Medicine*, **54**, 589.

Dahlin, D.C. (1949) Primary amyloidosis, with a report of six cases. *American Journal of Pathology*, **25**, 105.

Davidson, J.F., McNicol, G.P., Frank, G.L., Anderson, T.J. & Douglas, A.S. (1969) Plasminogen-activator producing tumour. *British Medical Journal*, **i**, 88.

Josselson, A.J. (1950) Amyloid disease of the heart. *Medical Clinics of North America*, **34**, 1137.

Kwaan, H.C. & Silverman, S. (1973) Fibrinolytic activity in lesions of hereditary haemorrhagic telangiectasia. *Archives of Dermatology*, **107**, 571.

Menon, I.S. (1967) Fibrinolysis in citrated and oxalated blood. *Lancet*, **i**, 116.

Osserman, E.F. (1965) *Immunopathology IVth International Symposium, New York*, p. 283. Grune and Stratton, New York.

Pick, A.L. & Osserman, E.F. (1967) *Proceedings of Symposium on Amyloidosis*, p. 100. Excerpta Medica Foundation, Amsterdam.

Redleaf, P.D., Davis, R.B., Kucinski, C., Hoilund, L. & Gans, H. (1963) Amyloidosis with an unusual bleeding diathesis. Observations on the use of epsilon amino caproic acid. *Annals of Internal Medicine*, **58**, 347.

Rowell, N.R. (1974) A painful bleeding syndrome associated with increased fibrinolytic activity. *British Journal of Dermatology*, **91**, 591.

Sirridge, M.S., Bowman, K.S. & Garber, P.E. (1958) Fibrinolysis and changes in fibrinogen in multiple myeloma. *Archives of Internal Medicine*, **101**, 630.

von Kaulla, K.W. (1963) In: *Chemistry of thrombolysis: human fibrinolytic enzymes*, p. 229. Charles C.Thomas, Springfield, Illinois.

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Electron microscopy of ancient Egyptian skin

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SUMMARY

Sections of skin were examined by electron microscopy from the sole of the foot of a 14-year-old Egyptian, who died 3200 years ago and was preserved naturally by desiccation. Remarkable ultrastructural preservation of the epidermal cells and their components was found.

During the summer of 1974 an autopsy was performed on 'Nakht' a 14-year-old weaver who died about 3200 years ago (Lewin *et al.*, 1974). He was not truly mummified but was preserved naturally by desiccation with his organs *in situ*.

Ultrastructural examination of the skin was undertaken to compare the findings with the original observations on truly mummified skin (Lewin, 1967).

MATERIALS AND METHODS

Skin samples were removed from the sole of the right foot and rehydrated in buffered formalin for 24 h. The specimens were then processed for electron microscopy. The rehydrated specimens were fixed in 6% glutaraldehyde in phosphate buffer (pH 7·4) for 2 h, followed by 1 h post fixation in 1% osmium tetroxide in phosphate buffer. The tissues were then embedded in Epon, and 1 μm sections were stained with toluidine blue for light microscopy. Ultrathin sections were cut from selected blocks and were stained with uranyl acetate followed by lead citrate.

RESULTS

Light microscopy showed good preservation of epidermal and dermal components of skin but cellular outlines were not observed.

At the ultrastructural level very good preservation of fine cellular components was observed. The epidermis particularly the keratinized layer (stratum corneum) was structurally intact (Fig. 1) and the intercellular junctions (desmosomes) were easily recognizable (Fig. 2). The basement membrane and the cytoplasmic processes of the basal epithelial cell layer (stratum basale) were reasonably well

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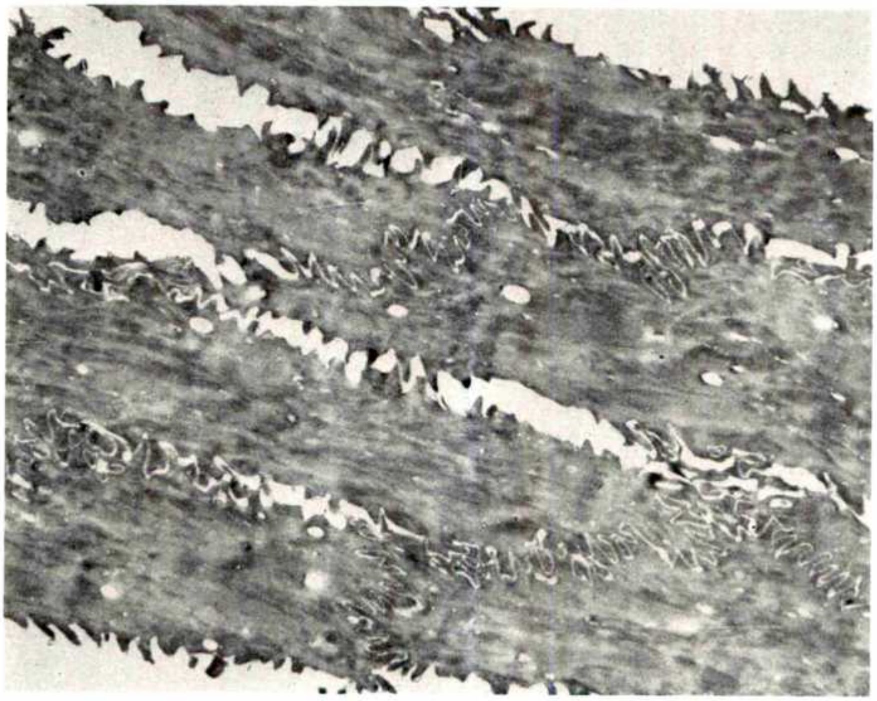

FIGURE 1. Low power electron micrograph of stratum corneum. The layering of cells and intercellular connections are well preserved (× 7000).

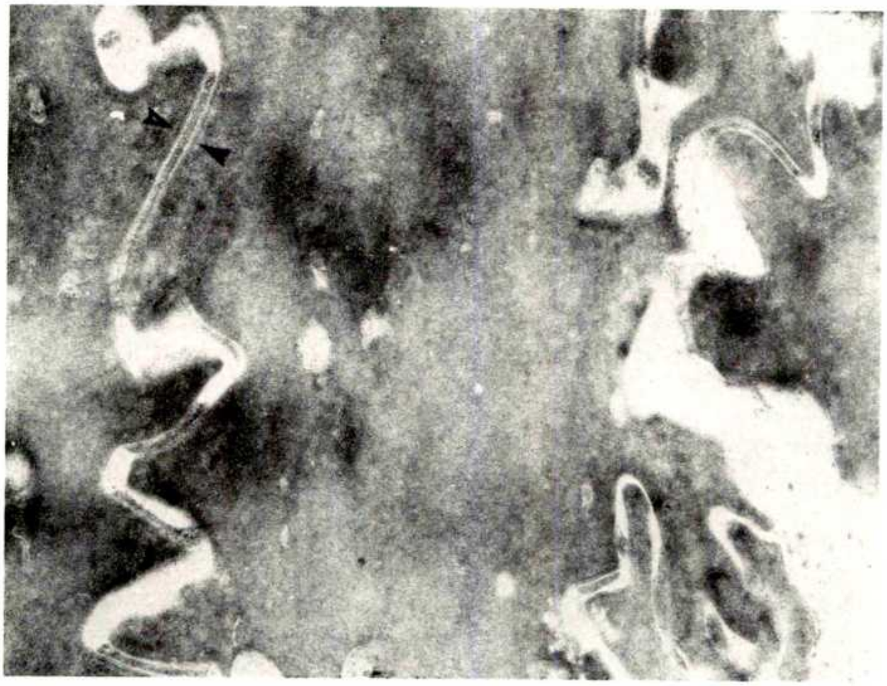

FIGURE 2. Portion of three cells of stratum corneum. Note preserved details of desmosomes (arrow heads). The wide intercellular spaces are probably artefacts (× 40,000).

preserved. In occasional epithelial cells of the stratum basale, outlines of the nucleus and cytoplasmic tonofilaments were found (Fig. 3).

The subepidermal tissues were composed of sheets of amorphous granular or fibrillar material in which collagen or elastica could not be identified. In some areas, oval cellular elements measuring 5–10 μm were observed (Fig. 4). These cells were characterized by large numbers of well-preserved mitochondria and large empty vesicles, but no other organelles were preserved.

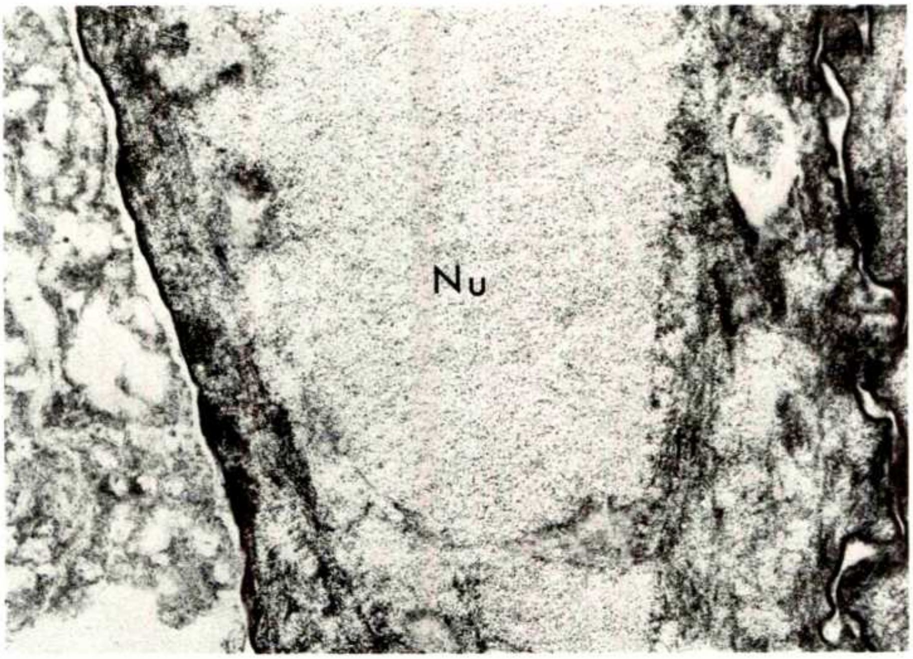

FIGURE 3. An epithelial cell showing the outline of a nucleus and cytoplasmic tonofilaments (tf) (× 30,000).

DISCUSSION

This study on the cellular components of skin preserved by natural desiccation confirms our previous reports on the ultrastructure of true mummified skin (Lewin, 1967).

Most of the cellular structures, particularly in stratum corneum, appeared morphologically intact and were comparable to freshly fixed material (Strauss & Matoltsy, 1973), except that no melanosomes were found.

Our original report on the ultrastructure of mummified skin included a description of cells, which were thought to be of dermal origin. In retrospect we believe that these structures represent bacteria or spores which are found in large numbers in mummified tissue.

The epidermal constituents of both mummified and desiccated skin were well preserved and therefore easily identified. On the other hand, the dermal components were mainly composed of amorphous fibrillar and granular material in which no collagen fibres were identified. The nature of cells within the dermis which contained many mitochondria could not be established. These cells may be preserved dermal cells or contaminating micro-organisms.

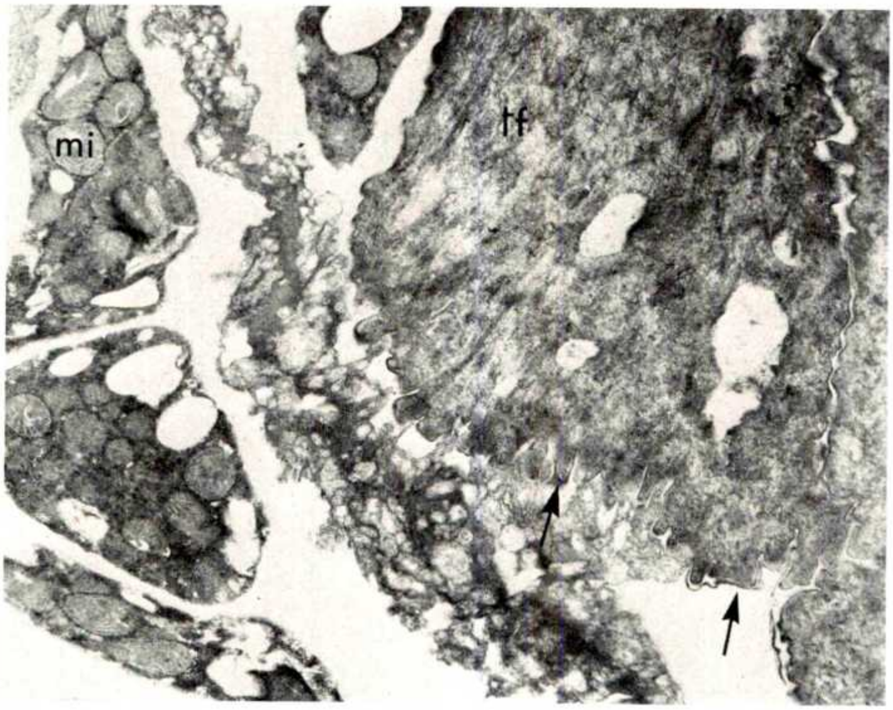

FIGURE 4. Basal cell layer (stratum basale) with basement membrane (arrows) and basal cytoplasmic processes. Note mitochondria (M) in cytoplasmic remains of cells in dermis (× 23,000).

Further examination of this type of material at ultrastructural level may be helpful in the study of the history of human skin diseases.

ACKNOWLEDGMENTS

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REFERENCES

LEWIN, P.K., MILLS, A.J., SAVAGE, H. & VOLLMER, J. (1974) 'Nakht', a weaver of Thebes. *Rotunda* (Journal of Royal Ontario Museum), **7,** 14.

LEWIN, P.K. (1967) Palaeoelectron microscopy of mummied skin. *Nature*, **213,** 416.

STRAUSS, J.S. & MATOLTSY, A.G. (1973) Skin. In: *Histology* (Ed. by R.O. Greep and L. Weiss) 3rd edn, p. 477. McGraw Hill, New York.

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Brief Communication

Absence of the sorbitol pathway in epidermis

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The sorbitol pathway offers a possibility for the conversion of D-glucose to D-fructose (Hers, 1960a) according to the reaction sequence:

Aldose reductase: $\text{glucose} + \text{NADPH} + \text{H}^+ \rightleftharpoons \text{sorbitol} + \text{NADP}^+$
Sorbitol dehydrogenase: $\text{sorbitol} + \text{NAD}^+ \rightleftharpoons \text{fructose} + \text{NADH} + \text{H}^+$
SUM: $\text{glucose} + \text{NADPH} + \text{NAD}^+ \rightleftharpoons \text{fructose} + \text{NADP}^+ + \text{NADH}$

Sorbitol dehydrogenase (SDH, EC 1.1.1.14) is widely distributed, the highest values being reported in liver (Gerlach & Hiby, 1974). Aldose reductase (AR, EC 1.1.1.21) is more limited in its distribution; the enzyme from seminal vesicles and placenta (Hers, 1960b) and that from the lens (Hayman & Kinoshita, 1965) have been studied in detail. Surprisingly, however, there seems to be no literature regarding this pathway in epidermis. We have therefore measured AR and SDH levels in this tissue, liver and lens of the same animal being assayed for comparison.

Guinea-pigs were killed by a blow to the head, shaved, and epidermis cut using a Castroviejo keratotome (0·2 mm). Samples of liver and lens were removed in the usual way. All specimens were homogenized in bovine serum albumin (BSA) solution (1 mg/ml), and centrifuged to yield a clear supernatant. Assay mixtures were as follows:

AR: tris (pH 6·2), 200 μmol; NADPH, 0·3 μmol; glucose, 600 μmol; tissue extract (Table 1). Total volume 3·0 ml.

TABLE 1. AR and SDH activities in guinea-pig tissues. The left column is in each case the concentration of tissue extract present in the reaction mixture (mg/ml), and the right column the calculated activity (μmol/min/g fresh weight)

Tissue	AR		SDH	
	Tissue conc.	Activity	Tissue conc.	Activity
Epidermis	10	0·015	10	Not demonstrated (<0·004)
Lens	3·3	0·43	3·3	0·082
Liver	3·3	0·13	0·33	3·9

SDH: tris (pH 8·6), 200 μmol; NAD$^+$, 1 μmol; sorbitol, 90 μmol; tissue extract (Table 1). Total volume 3·0 ml.

Blanks included cuvettes in which (a) the substrate was omitted, and (b) the tissue extract was replaced by BSA solution. In all cases the change in optical density at 340 nm was followed until a linear plot was established, and the net enzymic activity calculated as μmol conversion/min/g fresh weight of tissue.

Our results (mean of two animals) are shown in Table 1. The highest activity of AR was indeed seen in lens, and of SDH in liver. Despite the presence of a small but significant level of AR (apparently the first demonstration of this enzyme in epidermis), SDH was not found. It is clear, therefore, that the sorbitol pathway as a whole does not play a role in the carbohydrate metabolism of epidermis.

REFERENCES

HAYMAN, S. & KINOSHITA, J.H. (1965) Isolation and properties of lens aldose reductase. *Journal of Biological Chemistry*, **240**, 877.

HERS, H.G. (1960a) Le mécanism de la formation du fructose séminal et du fructose foetal. *Biochimica et Biophysica Acta*, 37, 127.

HERS, H.G. (1960b) L'aldose-réductase. *Biochimica et Biophysica Acta*, 37, 120.

GERLACH, V. & HIBY, W. (1974) Sorbitol dehydrogenase. In: *Methods of Enzymatic Analysis* (Ed. by H. V. Burgmeyer) 2nd English edn, Vol. 2, p. 569. Academic Press, New York.

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Comment

ESSENTIAL FATTY ACIDS AND THE SKIN

The essential fatty acids are a group of naturally occurring fatty acids of chain-length 18, 20 or 22 carbon atoms and containing between 2 and 6 methylene-interrupted unsaturated bonds, all in the *cis* configuration. There are two fundamental essential fatty acids from which all others are derived by metabolic chain elongation and desaturation. These are linoleic acid, giving rise to the ω-6 series, and α-linolenic acid, from which derives the ω-3 series (the ω-notation signifies the position of the first methylene-interrupted double bond numbered from the terminal methyl group of the acid). As their name implies, they are essential to the diet of man, indeed, of all higher animals, as they cannot be synthesized *de novo* from small molecular weight metabolites of carbohydrates and amino acids, as are all other lipids. Recent reviews comprehensively cover the chemistry and physiological function of the essential fatty acids in animals (Alfin-Slater & Aftergood, 1968; Holman, 1968, 1970) and man (Soderhjelm, Wiese & Holman, 1970). Relatively little, however, has been reported for skin specifically.

Fig. 1 illustrates interrelations of various essential fatty acids. All have been identified in the skin of man or laboratory animals (except ω-3 C18:4 and ω-3 C20:4) but some only in trace amounts (Vroman, Nemecek & Hsia, 1969; Jonsson & Anggard, 1972; Wilkinson, 1972; Prottey *et al.*, 1975, 1976). By far the most abundant essential fatty acids in skin are linoleic acid, derived solely from the diet, and arachidonic acid, derived from linoleic acid, or from traces in the diet. The various acyl decarboxylation and elongation enzymes that convert dietary linoleic or linolenic acid to the polyunsaturated fatty acids have been characterized in certain organs, mainly the liver (Alfin-Slater & Aftergood, 1968), but there is no conclusive evidence that the skin can perform these reactions independently. Rather, it must be assumed that although certain essential fatty acids are very important to the skin (e.g. arachidonic acid, as the precursor of prostaglandins) they originate from the liver via the plasma lipoproteins, and are not synthesized *per se* in the skin. Wilkinson (1972) has demonstrated labelling of various essential fatty acids in skin cultured *in vitro* with $[^{14}C]$-acetate but this is probably by acetate exchange, not synthesis *de novo*. Further research is necessary to establish whether the skin can perform any of the individual elongation and desaturation steps.

Function of essential fatty acids in skin

In the skin, as in all organs, essential fatty acids are principally found in phospholipids, and so have an accepted structural function in the lipoproteins of cell membranes and enzymes—their high degree of unsaturation bestowing fluidity or liquid crystallinity in these structures at physiological temperature, allowing conformational changes to occur. Essential fatty acids also occur in triglycerides (Carruthers, 1962), serving as energy stores or sebum precursors. Cutaneous sterol esters also contain essential fatty acids, but the function of these lipids in skin is equivocal (Yardley, 1969). A second and arguably more important function of some of the cutaneous essential fatty acids is as precursors of prostaglandins. Recent years have yielded an abundance of reports on the synthesis and role of prostaglandins in the skin, many of which have been cited in reviews (e.g. Greaves, 1972; Goldyne, 1975). Fig. 1 shows the three essential fatty acids concerned, namely, dihomo-γ-linolenic acid, that is converted to either PGE_1 or $PGF_{1\alpha}$ arachidonic acid, that yields PGE_2 or $PGF_{2\alpha}$ and ω-3 eicosapentaenoic acid, the precursor of PGE_3 or $PGF_{3\alpha}$. Of these three fatty acids arachidonic acid is the most abundant, Vroman *et al.* (1969) reporting that 9% of the total epidermal fatty acids of man is arachidonic acid. This does not presuppose that PGE_2 or $PGF_{2\alpha}$ are the most important cutaneous prosta-

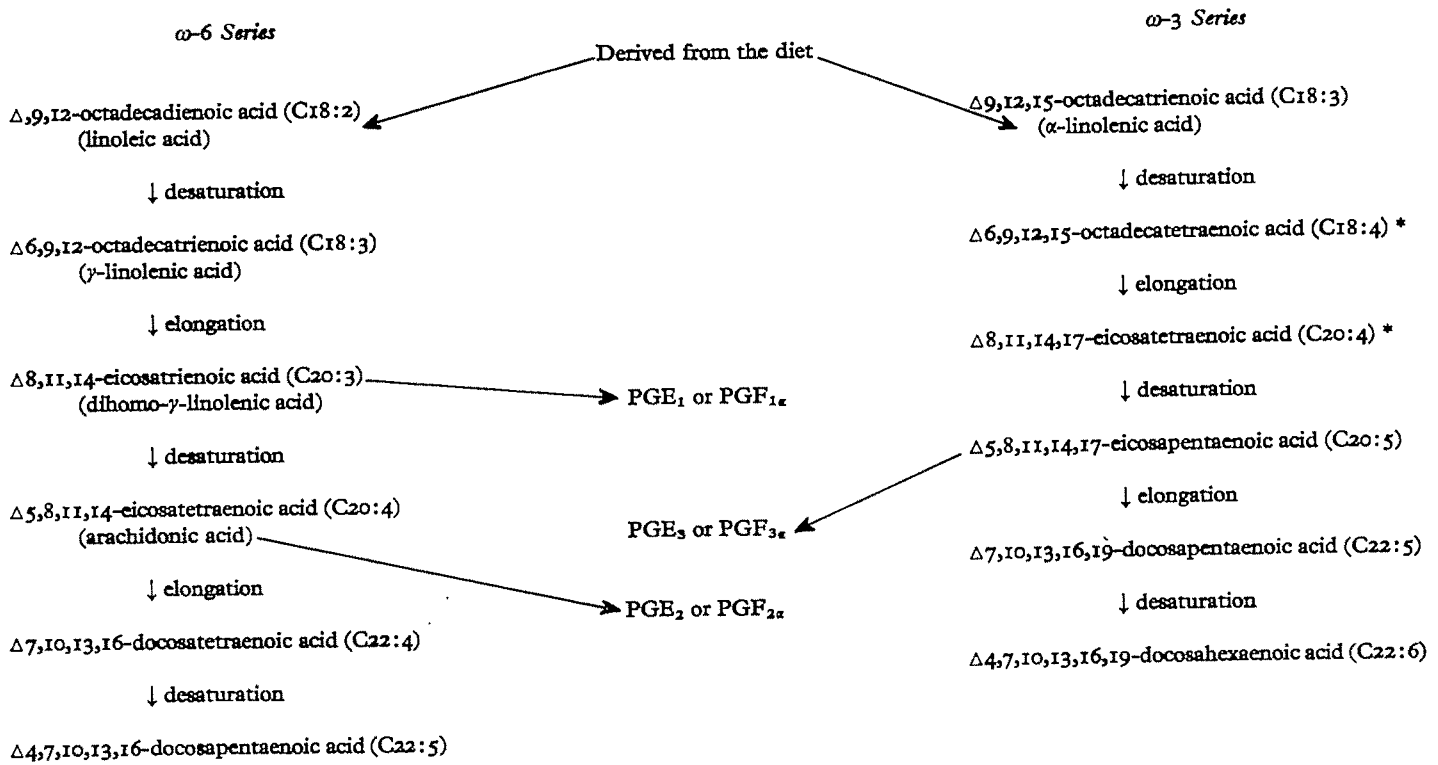
ω-6 Series
ω-3 Series
Derived from the diet
Δ,9,12-octadecadienoic acid (C18:2)
(linoleic acid)
↓ desaturation
Δ6,9,12-octadecatrienoic acid (C18:3)
(γ-linolenic acid)
↓ elongation
Δ8,11,14-eicosatrienoic acid (C20:3)
(dihomo-γ-linolenic acid)
↓ desaturation
Δ5,8,11,14-eicosatetraenoic acid (C20:4)
(arachidonic acid)
↓ elongation
Δ7,10,13,16-docosatetraenoic acid (C22:4)
↓ desaturation
Δ4,7,10,13,16-docosapentaenoic acid (C22:5)
PGE₁ or PGF₁α
PGE₃ or PGF₃α
PGE₂ or PGF₂α
Δ9,12,15-octadecatrienoic acid (C18:3)
(α-linolenic acid)
↓ desaturation
Δ6,9,12,15-octadecatetraenoic acid (C18:4) *
↓ elongation
Δ8,11,14,17-eicosatetraenoic acid (C20:4) *
↓ desaturation
Δ5,8,11,14,17-eicosapentaenoic acid (C20:5)
↓ elongation
Δ7,10,13,16,19-docosapentaenoic acid (C22:5)
↓ desaturation
Δ4,7,10,13,16,19-docosahexaenoic acid (C22:6)

FIGURE 1. Essential fatty acids of the skin.

Δ notation signifies positions of double bonds numbered from carboxyl terminal of acid; ω notation signifies position of first double bond, numbered from the methyl terminal of acid.

All acids shown (except those marked *) have been identified in human skin (Wilkinson, 1972) esterified to phospholipids.

glandins, however, as arachidonic acid has been shown to possess important structural properties in its own right (van Dorp, 1974). Despite advances in prostaglandin research we are still ignorant of the precise nature and cutaneous loci of those lipids (presumably phospholipids) that yield the fatty acid percursors of prostaglandins upon specific stimuli. It has been suggested that the sole function of essential fatty acids is to provide precursors of the prostaglandins (Beerthuis *et al.*, 1968), but this is not wholly acceptable (Holman, 1970), as some essential fatty acids are present in organs greatly in excess of the amounts of prostaglandins produced; skin is no exception.

Most published data concern the linoleic acid (ω-6) series of essential fatty acids. Although the linolenic acid (ω-3) series has been considered to be essential too, it cannot fulfil all functions of the ω-6 series, and so has been questioned as possessing true essentiality (Holman, 1970; van Dorp, 1974). None the less, skin contains the metabolic products of dietary ω-3 linolenic acid (eicosapentaenoate, docosapenta- and hexaenoate, Wilkinson, 1972), but we must await demonstration of their particular function in the skin.

Essential fatty acid deficiency and the skin of animals

Most research on the cutaneous function of essential fatty acids has concerned changes occurring in the skin as a result of dietary deficiencies of these acids. Burr & Burr (1929, 1930) reported that when rats were raised from weaning on a diet containing no essential fatty acids, their rate of weight gain was less than normally fed controls, despite constant caloric intake, and their skin became scaly on the hind paws and tail, and on the back the fur became clogged with dandruff-like flakes. This was followed later by alopecia. Such animals were also found to increase their intake of water, and this was later found to be due to a greatly increased rate of transepidermal water loss, not increased urine production (Basnayake & Sinclair, 1956). Indeed, the skin scaliness was found to be exacerbated by low ambient humidity (Brown & Burr, 1936) or restricted access to water (Thomasson, 1953). Basnayake & Sinclair (1956) found that when rats were placed on diets completely devoid of essential fatty acids immediately after weaning, the first change, after only a week or so, was the rapid reduction of linoleic and arachidonic acids in the skin lipids. After about 5 weeks these acids were virtually absent from the skin, and at this time animals would begin to gain weight more slowly than controls, to drink more water, and the skin scaliness developed. By about 10 weeks of the deficient diet the rate of transepidermal water loss began to rise rapidly to values about ten times that of normal rats. As the amounts of linoleic acid and arachidonic acid diminished in the skin the amount of $\Delta 5,8,11$ eicosatrienoic acid (ω-9) accumulated. This acid is normally present only in minute amounts in normal mammalian skin (Wilkinson, 1972), except when the amount of linoleic acid falls (as in the onset of essential fatty acid deficiency), in which case the enzymes that would normally convert linoleic acid to arachidonic acid instead convert oleic acid to the ω-9 eicosatrienoic acid (Fulco & Mead, 1959). This is not an essential fatty acid nor is it converted to a prostaglandin, despite its close similarity to the isomeric ω-6 eicosatrienoic acid, the precursor of PGE_1. This ω-9 acid is thought to be formed in order to sustain the overall degree of unsaturation in the membrane phospholipids in the absence of the essential fatty acids. The presence of this acid in appreciable quantities, usually, when the ratio of the trienoic acid to arachidonic acid is greater than 0·4 (Holman, 1960), is diagnostic for essential fatty acid deficiency.

Essential fatty acid deficiency with characteristic skin scaliness has been described in other species, such as dogs (Hansen, Sinclair & Wiese, 1954) and mice (Menton, 1968; 1970), raised from young on deficient diets. But it is difficult to induce the skin signs of essential fatty acid deficiency in adult animals by feeding a deficient diet, presumably because of the generally higher rate of metabolic activity in the rapidly growing young, and their lower body stores of essential fatty acids.

Microscopic skin changes in essential fatty acid deficiency have received close study. In rats, Williamson (1941) first described the epidermal thickening, in particular, the well-developed stratum granulosum and the thickened horny layer. Ramalingaswami & Sinclair (1953) described in greater detail the hyperplasia of the stratum basale and stratum granulosum, the latter exhibiting increased dark-staining keratohyalin. The stratum corneum was thickened but more closely packed and adherent than the open basket-like lattice of normal rats. Greater hyperplasia was seen around hair follicles which were often plugged with keratin. Sebaceous glands were initially hyperplastic, with more numerous and larger acini, but as the deficiency developed they became atrophic and the animals also became alopecic. In the upper dermis capillaries were more dilated, with increased numbers of mast cells and mononuclear cells. Kingery & Kellum (1965) confirmed many of these observations, in particular that hyperplasia and acanthosis occurred as quickly as 3 weeks after being placed on the deficient diet. Although bizarre hyperchromatic nuclei and frequent mitotic figures were seen in the basal layer as the deficiency progressed, parakeratosis was never observed. Histochemical staining revealed increased numbers of minute granules of phospholipid throughout the entire stratum corneum. As increased acid phosphatase activity throughout the stratum granulosum and stratum corneum was also observed, these authors suggested that phospholipid metabolism was deranged in essential fatty acid deficiency.

Menton (1968) described the following cutaneous changes in the essential fatty acid-deficient mouse: matting of fur, loss of skin elasticity, scaliness, alopecia, poor wound healing, erythema, lesions at mucocutaneous junctions, and lack of hair regrowth following plucking. Histologically, acanthosis of the basal layer resulted in increased thickening of the stratum granulosum and stratum corneum. There was increased mitosis and accompanying increased mitochondrial activity, assessed histochemically. In a later ultrastructural study Menton (1970) described a greater abundance of membrane coating granules in the differentiated cells, many of which had fused with cell membranes in the upper regions of the skin with extrusion of their contents. In the stratum corneum keratin filaments were more swollen and separated presumably reflecting a greater degree of hydration, but no parakeratosis was observed, although remnants of mitochondria, lipid droplets, vacuoles and unidentified vesicles were present. Menton suggested that the hyperplasia characteristic of essential fatty acid-deficient skin was due to a higher mitotic rate in the stratum basale, rather than to a decreased rate of desquamation.

The essential fatty acid-deficient rat is an unusual experimental model, and there is no direct counterpart in man, other than specific cases of fat malabsorption described below. As well as being useful in studies of cutaneous barrier function, essential fatty acid-deficient rats may also be valuable in experimental cutaneous inflammation. Prostaglandins are widely recognized as being important in the inflammatory process (Goldyne, 1975) as delayed vasoactivity mediators, as well as being thought to be involved in the regulation of various functions of leukocytes, such as histamine release from sensitized basophils, lysosomal enzyme release from neutrophils and in immune cytolysis (Bourne *et al.*, 1974). In the essential fatty acid-deficient rat there is markedly diminished prostaglandin synthetase activity, due to lack of substrate (arachidonic acid), and not due to inhibition or absence of the synthetase system (van Dorp, 1974). Therefore, these experimental animals may be useful for studying various aspects of cutaneous inflammation in which the participation of prostaglandins will be reduced or absent, otherwise only achieved by use of specific inhibitors (e.g. indomethacin). Bonta *et al.* (1974) have shown that carageenin oedema is reduced in intensity in essential fatty acid-deficient rats.

Essential fatty acid deficiency and the skin of man

There are no reported cases of naturally occurring essential fatty acid deficiency in man, but the condition may develop secondarily in disease after surgical or dietary intervention. Patients with chylous ascites maintained on low fat diets (Warwick *et al.*, 1959; Hansen & Wiese, 1944) developed dry scaly and eczematous skin lesions with accompanying lipid alterations. Holt *et al.* (1935) and Hansen *et al.* (1958) maintained infants on diets containing less than 0·1% calories as linoleic acid and essential fatty acid deficiency developed, with dry leathery skin and raw eczema in the intertriginous zones; in all these instances administration of linoleic acid caused the conditions to disappear. A common feature is that all instances concerned rapidly-growing infants. None the less, there are recent reports of essential fatty acid deficiency in adult man, with attendant skin abnormalities. Soderhjelm *et al.* (1970) and Caldwell, Jonsson & Othersen (1972) described patients from whom the small intestine had been removed and who had been maintained by intravenous feeding, in whom skin rashes or shiny, flaky skin resulted. The presence of ω-9 eicosatrienoic acid confirmed their status as essential fatty acid deficient. Furthermore, administration of linoleic acid healed the skin. Soderhjelm *et al.* (1970) and Paulsrud *et al.* (1972) have described skin lesions and abnormal lipids in essential fatty acid-deficient infants, resulting from intestinal resection and intravenous fat-free feeding. Collins *et al.* (1971) and Press *et al.* (1974) have separately described gut-resected patients who became essential fatty-acid deficient and developed skin scaliness. But parenteral administration of emulsions of soya lecithin, rich in linoleic acid, was effective. The skin of essential fatty acid-deficient man has recently received closer study (Prottey *et al.*, 1975). In three patients who had undergone massive intestinal resection and were unable to absorb dietary fat, very low concentrations of linoleic acid and arachidonic acid, but appreciable ω-9 eicosatrienoic acid, were detected in the epidermis. Skin scaliness was seen in two of the patients, but in all three transepidermal water loss was not elevated, unlike deficient rats (Prottey *et al.*, 1976).

Microscopically, the dry, scaly skin seen in essential fatty acid-deficient man shows similar derangement to that in deficient animals. Wiese (1971) has described acanthosis and hyperplasia of the epidermis, with thickened and denser stratum corneum, often with parakeratosis.

In acrodermatitis enteropathica, which is an hereditary disease characterized by bullous, pustular and eczematous skin lesions (illustrated in the review of Soderhjelm *et al.*, 1970), disturbances of plasma essential fatty acids have been reported. Cash & Berger (1969) found higher than usual amounts of linoleic acid in total serum lipids and the accumulation of ω-6 eicosatrienoic acid, but arachidonic acid was lower than normal. Oral administration of di-iodo hydroxyquinoline (Diodoquin), which rapidly reverses all aspects of the disease, was found to increase the proportion of arachidonic acid, but not fully. It is thought that the low proportion of this acid is not the major reason for this disease, as administration of arachidonic acid increased the amounts seen in serum without influencing the symptoms. Of many proposed theories of metabolic malfunction in acrodermatitis enteropathica (review by Nelder *et al.*, 1974) including abnormalities of zinc metabolism, the altered metabolism of essential fatty acids is one of the most ill-defined. It has been proposed by Cash & Berger (1969) that there is an enzymatic defect in the conversion of ω-6 eicosatrienoic acid to arachidonic acid. During remission, the abnormally high proportions of ω-6 eicosatrienoic acid are reduced. As yet, however, no investigations have been performed on the fatty acids of the clinically involved skin in acrodermatitis enteropathica. It may be suggested that alterations of prostaglandin synthesis and/or cutaneous barrier function may be manifested therein.

Restoration to normal of essential fatty acid-deficient skin

A feature of essential fatty acid deficiency is that it is rapidly reversible if essential fats are re-introduced either orally or parenterally. Burr & Burr (1929), Basnayake & Sinclair (1956) using rats, Hansen *et al.* (1954) with dogs, Collins *et al.* (1971), Caldwell *et al.* (1972) and Press *et al.* (1974b) with man, have all demonstrated that the various abnormal cutaneous parameters are rapidly restored by linoleic acid. In 1936, however, Shepherd & Linn demonstrated that topical application of essential fatty acids cured the caudal scaliness in rats. Essential fatty acids were collectively termed 'vitamin F' (Evans, Lepkovsky & Murphy, 1934), and rich sources suitable for cutaneous application included lard and vegetable oils. This practice was further developed by Grandel (1938) using concentrated preparations of 'vitamin F' which, after ten applications of as little as 40 μg linoleic acid, were claimed to prevent cutaneous signs of the deficiency in rats, requiring 20 times as much orally. As a result of these reports a large number of patents for creams, soaps, products alleviating dryness, accelerating wound healing, etc. and all containing 'vitamin F', appeared on the market.

Recently, cutaneous application as a means of reversing the cutaneous manifestations of essential fatty acid deficiency has been further utilized. Ziboh & Hsia (1972) have shown that topically applied PGE_2 effectively cured the scaly skin of essential fatty acid-deficient rats. Van Dorp (1974) suggested that cutaneous application of esters of linoleic acid or arachidonic acid or sunflower seed oil, a rich source of linoleic acid, reduced the elevated rate of transepidermal water loss in deficient rats. Prostaglandin E_2 had no effect, however. It has been shown (Prottey *et al.*, 1975) that cutaneous application of sunflower seed oil, but not olive oil (which contains little linoleic acid), lowered the rate of water loss, cured scaly lesions, and corrected the abnormal skin lipids of essential fatty acid-deficient patients. Also, cutaneous application of sunflower seed oil to these patients restored their abnormal plasma fatty acids, indicating that penetration of the linoleic acid through the skin had occurred (Press *et al.*, 1974a). There is evidence that linoleic acid plays a precise role in the epidermis in regulating barrier function. As well as reducing the elevated rate of transepidermal water loss in rats, when cutaneously applied as sunflower seed oil, there is also incorporation into the phospholipids of the skin (Prottey *et al.*, 1976). But not all of the essential fatty acids are equivalent in their ability to cure skin symptoms—some may repair impaired barrier function whereas others may cure the dermatosis. Mohrhauer & Holman (1963) showed that dietary arachidonic acid was better than linoleic acid at curing the deficiency, but ω-3 linolenic acid had only slight influence on the dermatitis. Pudelkewitz, Seuffert & Holman (1968) also found that dietary linoleic acid but not ω-3 linolenic acid could cure skin scaliness. We have found (Hartop & Prottey, in preparation) that cutaneous application of linoleic acid and ω-6 linolenic acid rapidly reduced the high rate of water loss in deficient rats, but ω-6 eicosatrienoic acid and arachidonic acid had no effect.

Speculative roles of essential fatty acids in skin

Various types of lipids and proteins are recognized as being involved in the maintenance of cutaneous barrier function (for example, see Matoltsy, Downes & Sweeney, 1968). Linoleic acid also plays an indisputable role, not only in the essential fatty acid-deficient rat, but also in other unrelated instances of impaired cutaneous barrier function. Jelenko, Wheeler & Scott (1972) showed that application of ethyl linoleate to experimentally burned rabbit skin caused a marked reduction of the elevated rate of transepidermal water loss, and Prottey *et al.* (1976) have shown that linoleic acid, topically applied as sunflower seed oil, rapidly lowers elevated water loss through the skin of rats exaggeratedly washed with detergent.

Although there is an obvious association of hyperkeratinization and elevated skin water loss (Felsher & Rothman, 1945), in essential fatty acid-deficient rats, at least, repair of barrier function and the healing of skin scaliness appear to be separable phenomena. In the author's experience (unpublished), although cutaneous application of linoleic acid will rapidly restore water loss to normal within 1 week, scaliness disappears only after 3 or 4 weeks. Ziboh & Hsia (1972) reported that the skin scaliness of essential fatty acid-deficient rats was cleared by application of prostaglandin E_2, but the author has found (unpublished) that topical prostaglandins E_1 and E_2 have no effect upon elevated water diffusion. These data suggest that of the various essential fatty acids (and prostaglandins derived from them), linoleic acid alone is important in the maintenance of normal barrier function. Presumably, this is due to the combination of linoleic acid in certain lipoproteins within the stratum corneum, but the precise nature of these awaits definition.

Unlike linoleic acid, a precise role of arachidonic acid in the lipids of skin has not been shown, even though it is as abundant as the former in man (Vroman *et al.*, 1969; Wilkinson 1972; Prottey *et al.*, 1975). As Ziboh & Hsia (1972) have shown that prostaglandin may reverse scaliness, and as prostaglandins have been suggested to be closely involved in the control of mitosis and differentiation by virtue of their interactions with cyclic AMP and cyclic GMP (Voorhees *et al.*, 1974), a role for arachidonic acid (and ω-6 eicosatrienoic acid) in regulation of epidermal homeostasis may be envisaged. These acids are known to be converted to prostaglandins in the epidermis. If one invokes such a role in regulation, one must also consider the types of lipids with which these acids are combined, and the location of these lipids in the epidermis where their effects are exerted, but these questions are unanswered at present. Moreover, it remains to be seen whether cutaneous essential fatty acids are involved in general instances of hyperkeratinization associated with impaired barrier function, such as psoriasis or ichthyosis (Grice & Bettley, 1967).

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REFERENCES

ALFIN-SLATER, R.B. & AFTERGOOD, L. (1968) Essential fatty acids re-investigated. *Physiological Reviews*, **48**, 758.

BASNAYAKE, V. & SINCLAIR, H.M. (1956) The effect of deficiency of essential fatty acids upon the skin. In: *Biochemical Problems of Lipids* (Ed. by G. Popjak and E. LeBreton), p. 476. Butterworth, London.

BEERTHUIS, R.K., NUGTEREN, D.H., PABON, H.J.J. & VAN DORP, D.A. (1968) Biologically active prostaglandins from some new odd-numbered essential fatty acids. *Recueil des Travaux Chimiques des Pays-Bas*, **87**, 461.

BONTA, I.L., CHRISPIJN, H., NOORDHOEK, J. & VINCENT, J.E. (1974) Reduction of prostaglandin-phase in hindpaw inflammation and partial failure of indomethacin to exert anti-inflammatory effect in rats on essential fatty acid deficient diet. *Prostaglandins*, **10**, 495.

BOURNE, H.R., LICHTENSTEIN, L.M., MELMON, K.L., HENNEY, C.S., WEINSTEIN, Y. & SHEARER, G.M. (1974) Modulation of inflammation and immunity by cyclic AMP. *Science*, **84**, 19.

BROWN, W.R. & BURR, G.O. (1936) Some recent studies on fat deficiency. *Journal of Biological Chemistry*, **114**, XVI.

BURR, G.O. & BURR, M.M. (1929) A new deficiency disease produced by the rigid exclusion of fat from the diet. *Journal of Biological Chemistry*, **82**, 345.

BURR, G.O. & BURR, M.M. (1930) On the nature and role of fatty acids essential in nutrition. *Journal of Biological Chemistry*, **86**, 587.

CALDWELL, M.D., JONSSON, H.T. & OTHERSEN, H.B. (1972) Essential fatty acid deficiency in an infant receiving prolonged parenteral alimentation. *Journal of Pediatrics*, **81**, 894.

CARRUTHERS, C. (1962) The fatty acid composition of dermal and epidermal triglycerides and phosphatides in mouse skin during normal and abnormal growth. *Cancer Research*, **22**, 294.

CASH, R. & BERGER, C. (1969) Acrodermatitis enteropathica: defective metabolism of unsaturated fatty acids. *Journal of Pediatrics*, **74**, 717.

COLLINS, F.D., SINCLAIR, A.J., ROYLE, J.P., COATS, D.A., MAYNARD, A.T. & LEONARD, R.F. (1971) Plasma lipids in human linoleic acid deficiency. *Nutritional Metabolism*, **13**, 150.

EVANS, H.M., LEPKOVSKY, S. & MURPHY, E.A. (1934) Vital need of the body for certain unsaturated fatty acids. *Journal of Biological Chemistry*, **106**, 431.

FELSHER, Z. & ROTHMAN, S. (1945) The insensible perspiration of the skin in hyperkeratotic conditions. *Journal of Investigative Dermatology*, **6**, 271.

FULCO, A.J. & MEAD, J.F. (1959) Metabolism of essential fatty acids. VII. Origin of 5, 8, 11 eicosatrienoic acid in the fat deficient rat. *Journal of Biological Chemistry*, **234**, 1411.

GOLDYNE, M.E. (1975) Prostaglandins and cutaneous inflammation. *Journal of Investigation Dermatology*, **64**, 377.

GRANDEL, F. (1938) Über Vitamin F. *Fette Seifen Anstrichmittel*, **46**, 150.

GREAVES, M.W. (1972) Prostaglandins and the epidermis. *British Journal of Dermatology*, **87**, 161.

GRICE, K.A. & BETTLEY, F.R. (1967) Skin water loss and accidental hypothermia in psoriasis, ichthyosis and erythroderma. *British Medical Journal*, **iv**, 195.

HANSEN, A.E., HAGGARD, M.E., BOELSCHE, A.N., ADAM, D.J.D. & WIESE, M.F. (1958) Essential fatty acids in infant nutrition III. Clinical manifestations of linoleic acid deficiency. *Journal of Nutrition*, **66**, 565.

HANSON, A.E., SINCLAIR, J.G. & WIESE, M.F. (1954) Sequence of histologic changes in skin of dogs in relation to dietary fat. *Journal of Nutrition*, **52**, 541.

HANSON, A.E. & WIESE, M.F. (1944) Clinical and blood lipid studies in a child with chylous ascites. *American Medical Association Journal of Diseases of Children*, **68**, 350.

HOLMAN, R.T. (1960) The ratio of trienoic:tetraenoic acids in tissue lipids as a measure of essential fatty acid requirement. *Journal of Nutrition*, **70**, 405.

HOLMAN, R.T. (1968) Essential fatty acid deficiency. In: *Progress in the chemistry of fats and other lipids* (Ed. by R.T.Holman), Vol. IX, pt. 2, p. 279. Pergamon Press, Oxford.

HOLMAN, R.T. (1970) Biological activities of and requirements for polyunsaturated acids. In: *Progress in the chemistry of fats and other lipids* (Ed. by R.T.Holman), Vol. IX, pt. 5, p. 611. Pergamon Press, Oxford.

HOLT, E., TIDWELL, H.C., KIRK, C.M., CROSS, D.M. & NEALE, S. (1935) Studies in fat metabolism. 1. Fat absorption in normal infants. *Journal of Pediatrics*, **6**, 427.

JELENKO, C., WHEELER, M.L. & SCOTT, T.H. (1972) Studies in burns. XI. Ethyl linoleate: the water-holding lipid of skin. B. Effects on *in vivo* burn eschar. *Journal of Trauma*, **12**, 974.

JONSSON, C.E. & ANGGARD, E. (1972) Biosynthesis and metabolism of prostaglandin E_2 in human skin. *Scandinavian Journal of Clinical and Laboratory Investigation*, **29**, 289.

KINGERY, F.A.J. & KELLUM, R.E. (1965) Essential fatty acid deficiency. *Archives of Dermatology*, **91**, 272.

MATOLTSY, A.G., DOWNES, A.M. & SWEENEY, T.M. (1968) Studies of the epidermal water barrier. Part III. Investigations of the chemical nature of the water barrier. *Journal of Investigative Dermatology*, **50**, 19.

MENTON, D.N. (1968) The effects of essential fatty acid deficiency on the skin of the mouse. *American Journal of Anatomy*, **122**, 337.

MENTON, D.N. (1970) The effects of essential fatty acid deficiency on the fine structure of mouse skin. *Journal of Morphology*, **132**, 181.

MOHRHAUER, M. & HOLMAN, R.T. (1963) The effect of dose level of essential fatty acids upon fatty acid composition of the rat liver. *Journal of Lipid Research*, **4**, 151.

NELDER, K.H., HAGLER, L., WIESE, W.R., STIFEL, F.B., LUFKIN, E.G. & HERMAN, R.H. (1974) Acrodermatitis enteropathica. A clinical and biochemical survey. *Archives of Dermatology*, **110**, 711.

PAULSRUD, J.R., PENSLER, L., WHITTEN, C.F., STEWART, S. & HOLMAN, R.T. (1972) Essential fatty acid deficiency in infants induced by fat-free intravenous feeding. *American Medical Association Journal of Clinical Nutrition*, **25**, 897.

PRESS, M., HARTOP, P.J. & PROTTEY, C. (1974a) The correction of essential fatty acid deficiency in man by the cutaneous administration of sunflower seed oil. *Lancet*, **i**, 597.

PRESS, M., KIKUCHI, H., SHIMOYAMA, T. & THOMPSON, G.R. (1974b) Diagnosis and treatment of essential fatty acid deficiency in man. *British Medical Journal*, **ii**, 247.

PROTTEY, C., HARTOP, P.J. & PRESS, M. (1975) Correction of the cutaneous manifestations of essential fatty acid deficiency in man by the application of sunflower seed oil to the skin. *Journal of Investigative Dermatology*, **64**, 228.

PROTTEY, C., HARTOP, P.J., BLACK, J.G. & MCCORMACK, J.I. (1976) The repair of impaired epidermal barrier function in rats by the cutaneous application of linoleic acid. *British Journal of Dermatology* (in press).

PUDELKEWICZ, C., SEUFFERT, J. & HOLMAN, R.T. (1968) Requirements of the female rat for linoleic acid and linoleic acids. *Journal of Nutrition*, **94**, 138.

RAMALINGASWAMI, V. & SINCLAIR, H.M. (1953) The relation of deficiencies of Vitamin A and of essential fatty acids to follicular hyperkeratosis in the rat. *British Journal of Dermatology*, **65**, 1.

SHEPHERD, M.I. & LINN, D.S. (1936) Evaluation of Vitamin F. *The Drug and Cosmetic Industry*, **38**, 629.

SODERHJELM, L., WIESE, M.F. & HOLMAN, R.T. (1970) The role of polyunsaturated acids in human nutrition and metabolism. In: *Progress in the Chemistry of Fats and Other Lipids* (Ed. by R.T.Holman) Vol. IX, pt. 4, p. 555. Pergamon Press, Oxford.

THOMASSON, H.J. (1953) Biological standardization of essential fatty acids. *International Review of Vitamin Research*, **25**, 62.

VAN DORP, D.A. (1974) Essential fatty acids and prostaglandins. In: *XXIVth International Congress of Pure and Applied Chemistry*, Vol. 2, p. 117. Butterworth, London.

VORHEES, J.J., DUELL, E.A., STAWISKI, M. & HARRELL, E.R. (1974) Cyclic nucleotide metabolism in normal and proliferating epidermis. *Advances of Cyclic Nucleotide Research* (Ed. by P. Greengard and G.A. Robison), Vol. 4, p. 117. Raven Press, New York.

VROMAN, H.E., NEMECEK, R.A. & HSIA, S.L. (1969) Synthesis of lipids from acetate by human preputial and abdominal skin *in vitro*. *Journal of Lipid Research*, **10**, 507.

WARWICK, W.J., HOLMAN, R.T., QUIE, P.G. & GOOD, R.A. (1959) Chylous ascites and lymphedema. *American Journal of Diseases of Children*, **61**, 317.

WIESE, H.F. (1971) Deficiency effects in human beings. In: *The Vitamins* (Ed. by W.H.Sebrell Jr and R.S. Harris), Vol., 3 p. 327. Academic Press, London.

WILKINSON, D.I. (1972) Polyunsaturated fatty acids of skin: identification and ^{14}C-acetate incorporation. *Lipids* 7, 544.

WILLIAMSON, R. (1941) A note on the epidermis of the rat on a fat-free diet. *Biochemical Journal*, 35, 1003.

YARDLEY, H.J. (1969) Sterols and keratinization. *British Journal of Dermatology*, **81**, Supplement 2, 29.

ZIBOH, V.A. & HSIA, S.L. (1972) Effects of prostaglandin E_2 on rat skin: inhibition of sterol ester biosynthesis and clearing of scaly lesions in essential fatty acid deficiency. *Journal of Lipid Research*, **13**, 458.

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Obituary

DR G.B.MITCHELL-HEGGS

With the passing of Dr Gordon Mitchell-Heggs on December 15th, British Medicine and particularly Dermatology has lost one of its most eminent and valued personalities. He entered St Mary's Hospital Medical School as a scholar and qualified in 1927 at the age of 23; he was made MRCP in 1933 and elected fellow in 1944. At St Mary's his quality was recognized by his appointment as Medical Superintendent, a post in which his talent for organization and co-operation became manifest. He was appointed in charge of the Skin Department in 1934 not so much on the basis of a formal training in dermatology, for he was only 30 (he studied in Vienna, Strasbourg and Budapest, the most notable centres in those days), but for his experience in general medicine, his promise, and his qualities of personal character. This was not unusual at that time, when detailed schemes of specialist training had not yet been formulated.

He was away in the RAMC through the entire duration of the War; his military hospital which he commanded, taking him to very varied theatres of war—BEF, Madagascar, Middle East, Sicily, Normandy and eventually Belgium. Here again his talent for organization and leadership came to the fore; he was mentioned in dispatches and in 1944 appointed OBE. Soon after the War the Royal College of Physicians of London organized a Conference of Physicians to which came eminent physicians from many countries as Europe started to find itself again. Gordon Mitchell-Heggs was the secretary of this conference.

When, in 1952, the British Association of Dermatology held the Tenth International Congress of Dermatology in London—the first revival of these congresses since 1945—Mitchell-Heggs was the natural choice for Secretary-General and a principal architect of this most successful meeting. His skill and fairness in negotiation, his wise judgment and his unfailing courtesy led to his election to the International Committee on Dermatology, of which he continued to be the British member for many years. The same qualities led to his appointment as Dean of St Mary's Hospital Medical School in 1960, a post which he held with distinction for 7 years.

In spite of these heavy committments he pursued an active clinical interest. His progressive thinking is evident in his contributions to Sir Alexander Fleming's pioneer book on Penicillin in 1946 and to W.S.C.Copeman's publication on Cortisone and ACTH in 1953. In 1950 he was the editor and major contributor of '*Modern Practice in Dermatology*'.

Apart from St Mary's his main dermatological appointment was to St John's Hospital for Diseases of the Skin which he held from the end of 1945 to his retirement in 1969; but he was also at various times appointed to the Royal Masonic Hospital, the Hospital of St John and St Elizabeth, the Royal Hospital, Chelsea, and St Luke's Hostel for Clergy. He was distinguished by presidency of the British Association of Dermatology, of St John's Hospital Dermatological Society, and of the Dermatological Section of the Royal Society of Medicine. He was active in the Society of Apothecaries and became Master.

Behind these notable achievements was a modest man of kindness, complete integrity and great courtesy. Loyal to his strict principles and to his colleagues, and to the institutions he served so faithfully, he will be remembered for his steadfast character and humane personality.

F.RAY BETTLEY

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Thesis

The Sebaceous Glands in the Vermillion Border of the Lips and in the Oral Mucosa of Man. Thesis presented by I.SEWERIN (1975) Royal Dental College, Copenhagen, Denmark. Copenhagen: Akademisk Forlag. Also published in *Acta Odontologica Scandinavica*, 33, Supplement 68, 226.

This thesis provides a thorough review of the literature not only on the sebaceous glands of the lip—first described by Morgagnus in 1706—and of the mouth—described by Fordyce in 1895—but also of free sebaceous glands (i.e. those not attached to a hair follicle) appearing elsewhere in the body. They may, for example, be found in the salivary glands, oesophagus, larynx, genitalia and in certain sites on the skin.

These glands are remarkable in that they have no exact counterpart in many animals, are visible to the naked eye, and differ in other important respects from ordinary sebaceous glands. They may contain Demodex even in the mouth.

In his study of the lips and mouth of 1717 persons, including 826 children, Sewerin found that 80% of adults have macroscopically visible free sebaceous glands on the lips, and 95% in their mouth. The different sites are carefully mapped out. There was a lower incidence in children. The lesions tend to be grouped, usually less than 1 mm across, and are seldom very numerous. These figures are comparable to those in the literature. It is argued that the word ectopic cannot rightly be used for something which is so uniformly present, but should be applied to glands found, for example, in the larynx or uterus. However, fully formed pilo-sebaceous follicles are indeed ectopic in the mouth.

These mucosal sebaceous glands seem to have no function but are perhaps rudimentary remnants of glands which originally acted as scent glands. The glands have not been seen in fetuses, but have occasionally been noted in the new-born. In this study the author is able to show a moderate stimulatory effect of puberty on the development of the glands but maximum development does not occur until a decade or so later. These glands seem to give rise to relatively little pathology. In this series he noted two comedones, twelve pseudocysts and three dilated excretory ducts.

Book reviews

Histopathology of the Skin. W.F.LEVER and G.SCHAUMBURG-LEVER (1975) 5th edn. Philadelphia. Toronto: J.B. Lippencott Company. Pp xx+793, illustrated. Price £36.40.

This book needs no introduction to dermatologists and pathologists. Previous editions have been the standard reference book on skin pathology in most laboratories and libraries in the English-speaking world for over a quarter of a century, and have been valued for their clear, concise descriptions of the various diseases and for their up-to-date and comprehensive references. The fifth edition retains the virtues of its forerunners in these respects and has been enlarged by the descriptions of newly recognized diseases and by giving more weight to histochemistry, electron microscopy, immunology and other newer methods of studying disease processes. The emphasis has thus changed somewhat from the almost purely descriptive approach of the earlier editions, although it is not possible in a book even of this size to give adequate attention to the contemporary ideas on pathological mechanisms as they influence histogenesis. The authors have probably reached a compromise which will be satisfactory for the practical histopathologist engaged in reporting skin sections.

The book is open to criticism on the quality of its illustrations. They have been chosen with apparent care to illustrate important aspects of the subject which are difficult to describe, but they are technically mediocre. There is a murky quality, with evidence of uneven illumination in some, and few possess the clear, crisp definition which is achieved by the best contemporary publications of photomicrographs. The same criticism can be levelled at the electron micrographs which are grouped at the end of the book. A justification for their grouping would be that some readers are unfamiliar with the EM appearances of the skin and its diseases and will thus be helped to understand the subjects by having them as a series by themselves. The contrary view, that their only justification in a book of this type is to illuminate the histopathology, would have demanded that they be placed in the appropriate section. Had this view been taken their number would probably have been reduced.

The book is well produced and sturdy, designed to stand up to much use. The index is good. The price has increased greatly on the fourth edition. It will however remain as indispensable as previous editions and can be commended for its clear, judicious and comprehensive account of the subject.

K.V.SANDERSON

The Pharmacological Basis of Therapeutics. LOUIS S.GOODMAN and ALFRED GILMAN (1975) 5th edn. London: Baillière Tindall. Pp. 1704, 90 illustrations. Price £18.00.

The editors have maintained and strengthened the close correlation between pharmacology and clinical medicine, characteristic of previous editions. More than one third of the contributions have been either rewritten or thoroughly revised and the new chapter on pharmacokinetics is very impressive, with good illustrations. The central role played by the adenyl cyclase–cyclic AMP system in the mediation or regulation of many drug effects now receives full recognition. Although, like previous editions, the new one makes only perfunctory mention of many of the more widely used topical agents, there is a great deal of useful information for the dermatologist. The chapter on cytostatic agents including methotrexate is good with valuable references. The section on anti-viral agents is disappointing. There are new sections on histamine H_2 receptors and H_2 receptor blocking agents and on prostaglandins and derivatives—drugs which will become increasingly familiar to dermatologists over the next few years. The new edition should be available for reference in every skin department.

M.W.GREAVES

Iconographia Selecta Dermatohistologica. Ein Dermatohistologgischer Atlas. L.SZODORAY and K.VEZEKENIJI (1975) Budapest: Akademiai Kiado. Pp. 296, 432 illustrations.

What does one expect from a small illustrated text book of skin pathology? Unfortunately there is no single answer. The dermatologist in training needs rather more text and key references than the general pathologist who wants to make diagnoses on unfamiliar morphological patterns and requires many and logically arranged (for him) photomicrographs. The practising dermatologist needs an amalgam of both the above and in addition he requires the text to refer frequently to clinical situations. Clearly, the task of producing such a book is extraordinarily difficult. It is made even more difficult by the absolute necessity of providing good illustrations; without photomicrographs that adequately demonstrate the essence of the disease process under discussion the whole production loses impact and any real value. This book by Professor Szodoray and Dr Vezekeniji from Budapest tries to encompass the whole of skin pathology in less than 300 pages by using copious photomicrographs and a minimum of text. It is a brave attempt but loses somewhat because the illustrations do not often succeed in bringing the reader's eyes to the 'nitty gritty' of the processes under discussion. The grouping of the conditions described is perhaps somewhat unexpected. Why for example are psoriasis, lichen planus, lichen moniliformis, plasma cell balanitis and Fox Fordyce disease discussed in the same section? The German text should not deter those English dermatologists who payed attention while their schoolmasters explained the mysteries of German grammar.

R.MARKS

Progress in Allergy. Edited by P.KALLOS, B.H.WAKSMAN and A. DE WECK (1975) Vol. 19. Pp. xx+312, 19 figures, 29 tables. Price US $49.25.

The 19th volume of *Progress in Allergy* follows soon after its predecessor and again maintains a high standard of review articles written in considerable depth, mainly perhaps for the immunologist and together providing a thousand references. This year there are several articles likely to be of interest to dermatologists. In the first chapter, Ford reviews lymphocyte migration. There follows an authoritative account of the properties of IgE by the Ishizakas. A long paper on IgE in animals takes us well away from the concept of Coca that atopy was a peculiarly human disease, although we are still some way from having an animal model exactly comparable to human atopic dermatitis. Fitch then reviews the various mechanisms by which selective suppression of immune responses can be achieved. The outstanding example of the exploitation of this is the prevention of Rhesus disease. Hopefully other examples will one day allow more elegant treatment than the present blunderbuss methods of immunosuppression.

Although few dermatologists will need to buy this volume any interested in immunology will want to refer to it.

R.H.CHAMPION

British Journal of Dermatology (1976) **94**, 592.

News and Notices

Symposium on Differential Diagnosis in Dermatopathology

A Symposium 'Differential Diagnosis in Dermatopathology' directed by Dr A.Bernard Ackerman and sponsored by the Departments of Dermatology and Pathology of New York University School of Medicine will be held at New York University Medical Center from 1.30, Thursday, 14 October to 12.30 Saturday, 16 October, 1976. The Symposium focuses on forty-two of the most difficult problems of differential diagnosis in dermatopathology. The crucial diagnostic differences, histological and clinical, will be graphically presented in 5 minutes, followed by discussion in the form of dialogue between the audience and the faculty. The faculty includes Drs Edward Wilson Jones, Hermann Pinkus, Robert Freeman, Lewis Shapiro, Arkadi Rywlin, John Maize, David Silvers, and A.Bernard Ackerman. Dr Wilson Jones will also give the Howard Fox lecture.

Further details may be obtained from the Office of the Associate Dean, New York University Post-Graduate Medical School, 550 First Avenue, New York, N.Y. 10016, U.S.A.

2nd National Course on Advances in Biological Research Applied to Dermatology

This course will be held under the auspices of the Italian Society of Dermatology and Syphilology and of the European Society for Dermatological Research.

The preliminary programme will include: Innervation of the skin; Horny layer and its functions; Sex hormones and skin; The flora of the skin; Advances in virus diseases of the skin; Immunological aspects of melanoma; Advances in psoriasis; Clinical and pathogenetic aspects of cutaneous precanceroses; Fibrous proteins of the skin.

The course will be held in the Cairoli College, Piazza Cairoli, Pavia, from 14 to 18 September 1976. Italian and foreign lecturers will be members of the Faculty. Members of the Faculty and participants will be accommodated in the College. Registration will be open to a limited number of graduates, deadline 31 July 1976. Course fee, Lire 50·000.

For further information write to Dr D.Cerimele, Clinica Dermatologica dell'Università, Policlinico S. Matteo, 27100 PAVIA, Italy (Phone 21633).

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	µg	cubic millimetre(s)	mm^3	micrometer(s)	µm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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References 1. Whitefield, M. and McKenzie, A. W., Brit. J. Derm. (1975), **92**, 585. 2. Fredriksson, T. and Gip, L., Dermatology Digest. (1976) **Jan.** 27. 3. Barry, B. W. and Woodford, R., Brit J. Derm. Accepted for publication.

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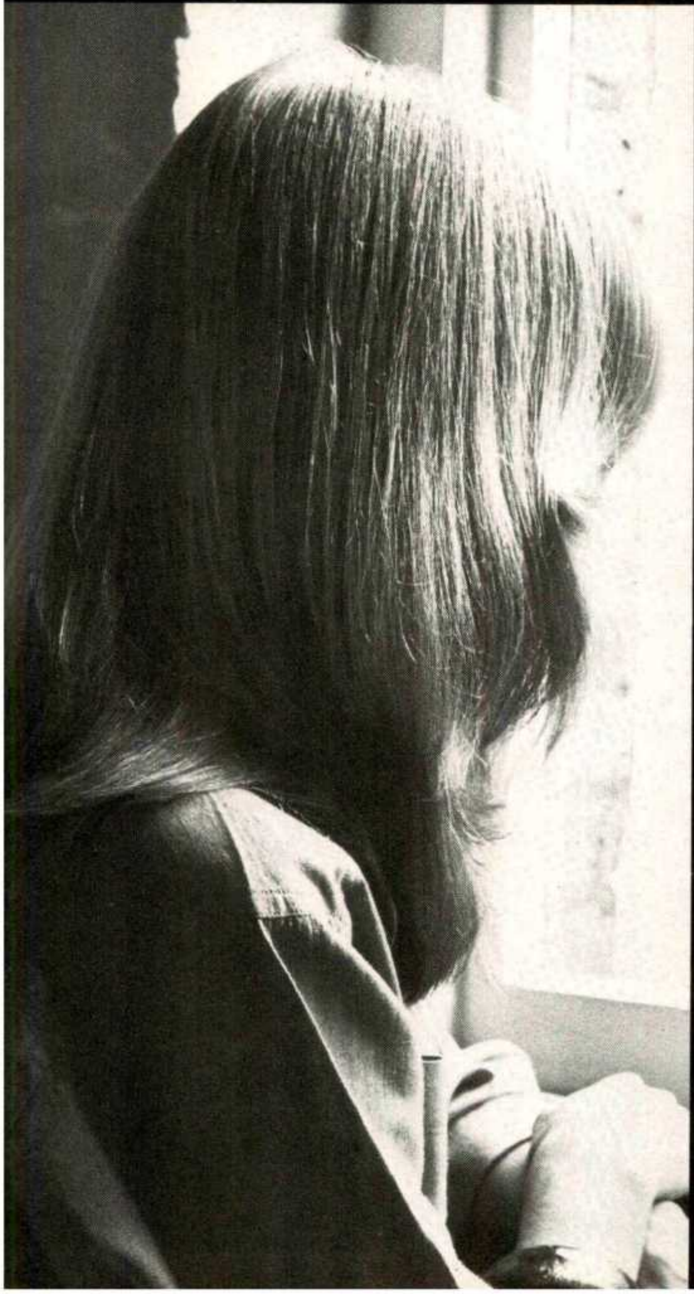

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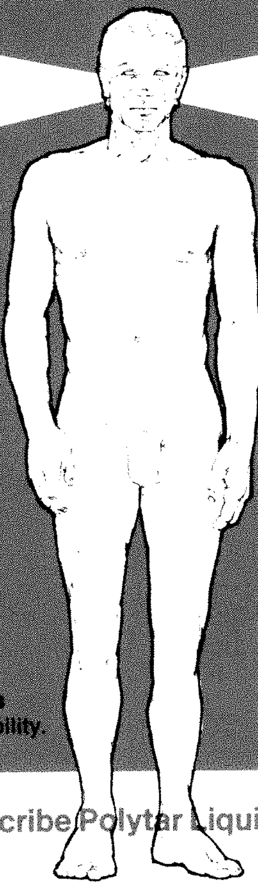

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Practitioner*, (1974), **213, 864.

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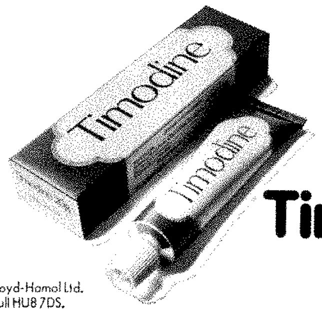

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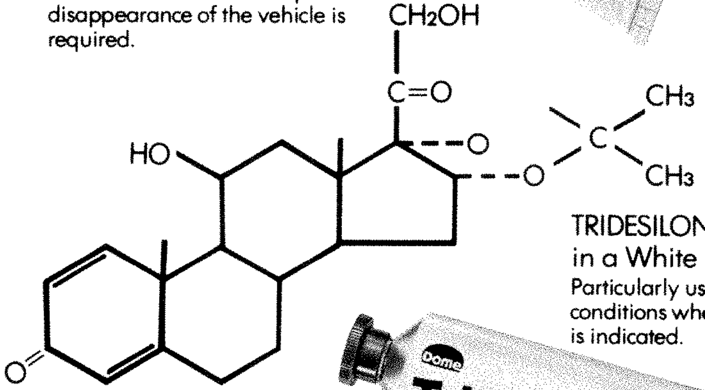

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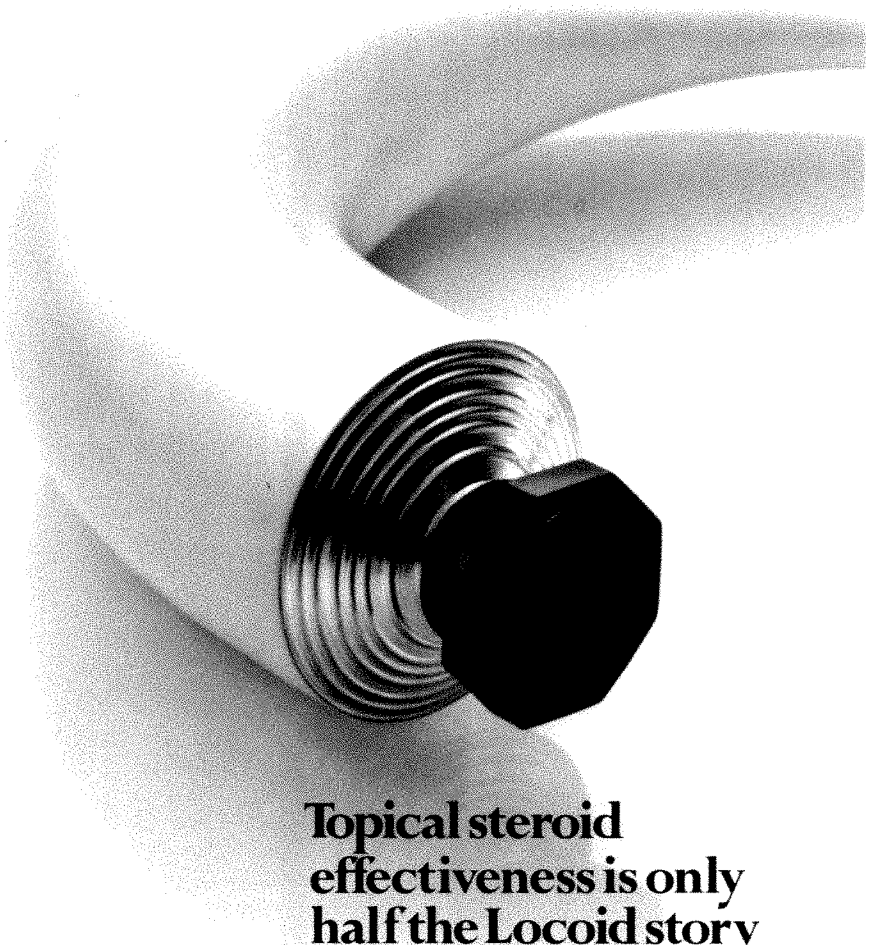

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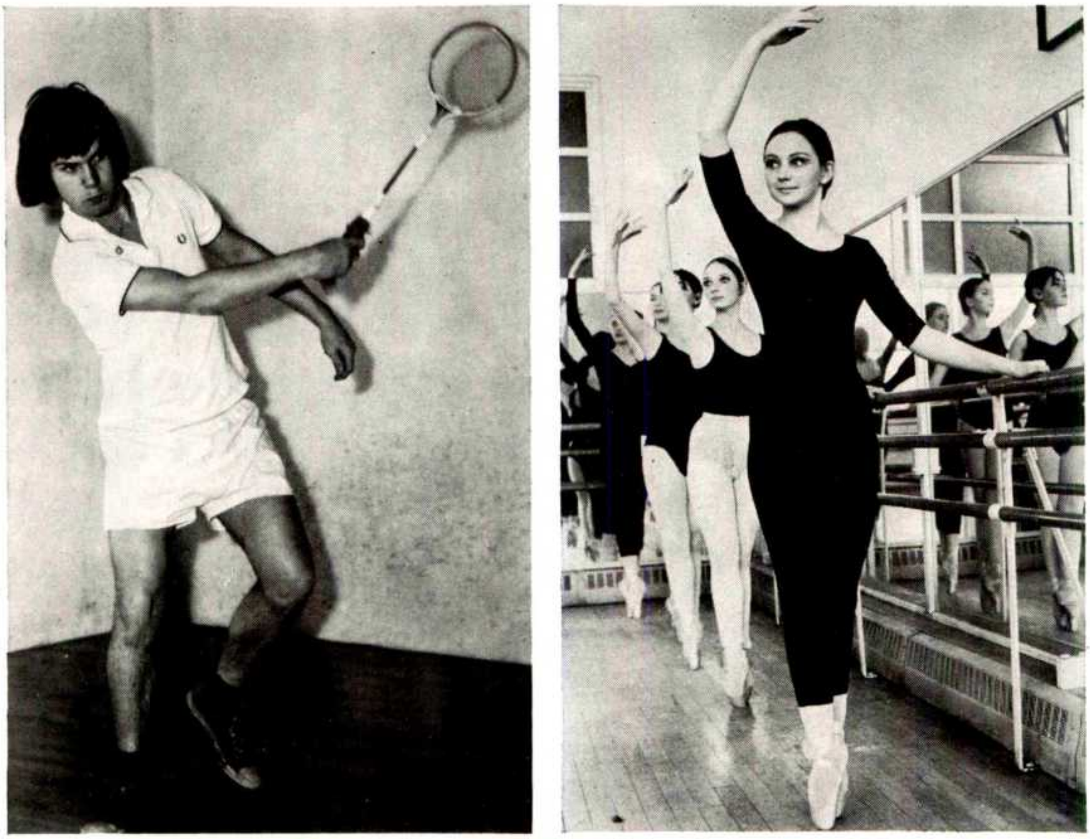

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References:
1. Bunney, Mary H. (1971), Practitioner, **207**, 197.
2. Pegum, J. S. (1972), Practitioner, **209**, 453.
3. Bunney, Mary H. (1974), Prescribers Journal, **14**, 118.

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Clinical and Laboratory Investigations

Clinical patterns and results of radioallergosorbent test (RAST) and skin tests in penicillin allergy

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SUMMARY

Seventy-nine patients with acute or former reactions to penicillin were investigated by a benzylpenicilloyl (BPO)-specific RAST and/or by skin tests with penicilloyl-polylysine (PPL), benzylpenicillin and penicilloic acid and the results were correlated with the different clinical pictures. Positive RAST and skin test results could be found in patients with anaphylactic shock, urticaria and serum sickness-like reaction and sometimes in a special group of exanthems, which are characterized by the existence of many different lesions at the same time, therefore called 'polymorphic exanthems', and often observed after high-dosage penicillin therapy. In cases of scarlatiniform or morbilliform exanthems no positive results were found. The BPO-specific RAST showed an overall correlation of 95·1% with skin tests using PPL. However, some patients with positive skin tests to benzylpenicillin and penicilloic acid did not have detectable circulating IgE antibodies to BPO. This emphasizes the need for including these antigens in *in vitro* methods.

The RAST was informative even at the time of the allergic reaction or in the first 15 days afterwards and seems to be very valuable for early diagnosis of penicillin allergy especially in cases when many drugs have been given.

Allergic reactions are severe complications of antibiotic therapy and the diagnostic problems in these situations have not yet been satisfactorily solved. Many factors are involved and it is therefore likely that the use of different diagnostic methods at the same time will be of help in the choice of further antibiotic treatment (de Weck, 1972; Girard, 1974; Vickers & Assem, 1974).

In penicillin allergy, reactions of the immediate type, like anaphylactic shock and urticaria, often occur and reaginic antibodies (IgE) are likely to be involved (Stewart & McGovern, 1970). In such cases, skin tests with different antigens (penicilloyl-polylysine for major determinant and sodium penicillin G and penicilloic acid for minor determinants testing) have been found to be useful (de Weck, 1965; Levine & Zolov, 1969; Schneider, 1974). However, because these are time consuming and carry a definite risk of generalized reactions, there is a need for *in vitro* tests which can replace the skin tests. Previous investigations have shown that the RAST (Radioallergosorbent test) could work as an alternative or complementary method to the skin tests (Wide & Juhlin, 1971; Juhlin & Wide, 1972; Wide, 1973).

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The purpose of this investigation was to study the correlation between results of RAST and skin tests in relation to clinical patterns in patients with penicillin allergy. Minor determinants were included in the skin tests to investigate their importance. Can the *in vitro* test RAST be diagnostically informative even at the time of the allergic reaction? This was studied by following the serum levels of specific IgE antibodies and total IgE during the first week and in some cases during the first months after the reaction.

PATIENTS

Seventy-nine patients (36 females, 43 males) aged from 7 to 75 years (average 41·05 years) were investigated. They have been observed with clinical symptoms after penicillin therapy in the 2nd Department of Dermatology, University of Vienna, or during consultant visits at other University hospitals in Vienna (Clinics of Internal Medicine, Surgery, Neurology). According to the date of the drug reaction and the performance of diagnostic tests they were divided into three groups:

Group A. Patients seen during the first 24 h of acute reactions to penicillin and tested with the available test systems including skin tests later on.

Group B. Patients with a history of reactions to penicillin 18 days to 11 years previously and tested by the available test systems including skin tests.

Group C. Patients seen during the first 24 h of acute reactions to penicillin, but tested by *in vitro* methods only.

MATERIALS AND METHODS

Sera

Blood samples were drawn from each patient at the time of the clinical manifestations and/or at the time of skin testing. After clotting for 2 h at room temperature the sera were separated and stored at −25°C until the *in vitro* tests were performed.

Radioallergosorbent test (RAST)

The RAST technique (Wide, Bennich & Johansson, 1967) was used to detect IgE antibodies to benzylpenicilloyl (BPO). One ml of Cilligen PPL-50 (Sigma) containing about 50×10^{-5} M BPO in saline phosphate buffer was mixed with 400 mg CNBr-activated microcrystalline cellulose particles (Wide, 1969) during the coupling procedure. The suspension of PPL cellulose conjugates was added in 0·5 ml aliquots to 0·05 ml serum for each test. Both steps of incubation were made under vertical rotation. The results were considered as negative when the activity was less than mean + 2 s.d. for negative controls. The degree of positive reactivity was expressed in sorbent units (SU) (Stenius & Wide, 1969).

Serum IgE levels

The determination of the IgE concentrations in the sera was made with Phadebas IgE test (Pharmacia AB, Uppsala, Sweden) which is based upon the radioimmunosorbent technique (RIST) (Wide, Axén & Porath, 1967) applied for the assay of IgE (Johansson, Bennich & Wide, 1968).

Passive haemagglutination

Passive haemagglutination was carried out according to Levine, Fellner & Levytska (1966a) and Levine *et al.* (1966b), as previously reported (Kraft *et al.*, 1971; Stemberger *et al.*, 1971). Briefly, human Rh pos. O red blood cells were coated with benzylpenicilloyl (BPO) groups following incubation with sodium penicillin G at pH 9·7 and using a mixture of dextran-normal rabbit serum-tris-buffered saline as diluent. The 'titre' was recorded as the highest dilution giving a reaction with many small clumps and little or no free cells (+ + in Levine's classification).

Skin-tests

The tests were performed with different antigens and different concentrations on the inner side of the forearms. The following reagents and procedures were used:

(a) Benzylpenicilloyl-polylysine (PPL),* first used for scratch test at a concentration of 500 nmol BPO/ml and followed if negative by intradermal tests (0·005 ml) with 25 and 250 nmol BPO/ml.

(b) Benzylpenicillin G,* first used for scratch test (200,000 U/ml) and followed if negative by successive intradermal injection (0·005 ml) of 10, 100 and 1000 U/ml.

(c) Benzylpenicilloic acid,* used for scratch and intradermal (0·005 ml) tests at a concentration of 0·5 mg/ml.

(d) Ampicillin (Binotal), used for scratch and intradermal (0·005 ml) tests at a concentration of 100 mg/ml.

The diameters of weal and flare reactions were measured after 20 min and the reaction was considered positive when the diameter was greater than 5 mm. Controls with isotonic NaCl solution and in doubtful cases with a histamine solution (10 mg/ml for scratch and 0·1 mg/ml for intradermal tests) were used.

RESULTS

Clinical data

The clinical patterns of the observed drug reactions are listed in Table 1. There was no difference between the clinical patterns in groups A, B and C except for the different times elapsing between the appearance of the drug reactions and the carrying out of skin tests. Urticaria and polymorphic exanthems were the most frequent manifestations, but anaphylactic shock, serum sickness-like reaction, and scarlatiniform or morbilliform exanthems also often occurred. The last-mentioned manifestation (Fig. 1a) was distinct from the polymorphic exanthem which is characterized by large macular or maculopapulovesicular, often together with haemorrhagic, lesions occasionally resembling erythema multiforme (Fig. 1b).

Anaphylactic shock was observed within 1 h after penicillin administration, urticaria between a few hours and 4 weeks, serum sickness-like reactions, and scarlatiniform, morbilliform or polymorphic exanthems between a few days and 5 weeks (in most cases in the first week).

Possible sources of the drug reactions were all kinds of commercially available penicillins such as sodium penicillin G, procaine penicillin G, clemicol penicillin G, penicillin V, cloxacillin, ciclacillin, amoxycillin, carbenicillin and ampicillin. Patients who had anaphylactic shock had been treated previously with sodium penicillin G, penicillin V or depot-penicillins, whereas patients with urticaria and serum sickness-like reactions were mostly under treatment with depot-penicillins. Nine of twenty patients with polymorphic exanthems were treated with high doses of penicillin (2×10^6 U sodium penicillin G/day) in combination with cloxacillin (5), ciclacillin (3), ampicillin (1) and 4 with ampicillin only.

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* The test substances were kindly supplied by Professor Dr A.L. de Weck, Bern and Hoffman La Roche, Basel.

TABLE 1. The clinical patterns of drug reactions after penicillin therapy (79 patients)

	Groups		
	A	B	C
Anaphylactic shock	5	5	3
Urticaria	6	18	6
Serum sickness-like	4	1	0
Scarlatiniform or morbilliform exanthem	3	2	0
Polymorphic exanthem	12	3	5
Erythema nodosum	1	0	0
Haemolytic anaemia	0	0	1
Pruritus	0	1	0
Questionable	0	3	0
Total 79	31	33	15

RAST and skin test results

Eleven patients with acute reactions after penicillin therapy (group A) and twelve patients with a history of reactions to penicillin (group B) gave positive skin tests with 'major and minor determinants' within 20 min after challenge (Table 2). By means of the BPO-specific RAST only eighteen patients of these groups and six patients of group C showed specific IgE (including weak positive results). Eight cases with positive skin tests to 'minor determinants' did not give a positive BPO-specific RAST but on the other hand three skin-test negative patients did give positive RAST results. Combining both methods brought up the number of patients with 'proved' penicillin allergy to a total of thirty-two. The three additional patients, only reacting in RAST, had typical case histories (2 urticaria and 1 anaphylactic shock after penicillin injections). The BPO-specific RAST showed an overall correlation of 95·1% with PPL performed skin tests, and patients with a 'major determinant' positive skin test always had a positive RAST.

Nineteen of twenty-three patients with positive skin tests and nineteen of twenty-four patients with positive, or weak positive, RAST had symptoms of immediate type reactions such as anaphylactic

TABLE 2. Correlation of RAST and skin-test results in penicillin allergy

	RAST (major determinant specific)			Skin-tests (major and minor determinants specific)
	+	(+)	−	
Group A	7	0	4*	+
(31 patients)	2	0	18	−
Group B	5	3	4*	+
(33 patients)	0	1	20	−
Group C	5	1	9	ND
(15 patients)				

* Minor determinants positive skin tests only.

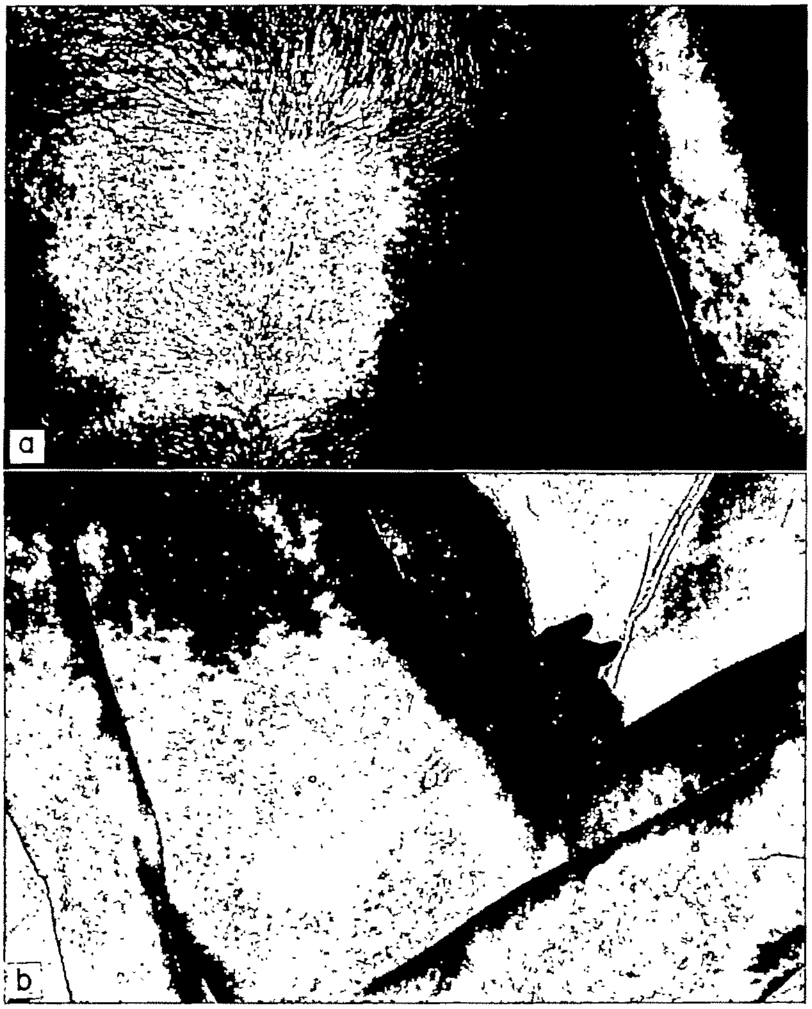

FIGURE 1. Different exanthematic eruptions after penicillin therapy: morbilliform (a) and polymorphic (b) exanthems.

shock, urticaria and serum sickness-like reactions. With regard to these clinical patterns, seven of fifteen patients in group A and twelve of twenty-four patients in group B had a positive skin test and eleven of twenty-four patients in groups A and C and nine of twenty-four patients in group B had a positive or weakly positive RAST. In cases of morbilliform exanthems no positive results in skin test or in RAST were found. Between these extremes, of twenty patients with polymorphic exanthems three had positive skin tests and five had positive RAST. One patient with erythema nodosum showed a positive skin test with 'minor determinants', but no reactivity was found in the RAST.

Specific IgE levels assayed by RAST

Sera from patients with positive RAST varied in 'reaginic antibody activity', sometimes increasing, sometimes decreasing after the first serum sample (Fig. 2). But there was no obvious correlation between the strength of the RAST result (sorbent units) and the severity or the type of the allergic

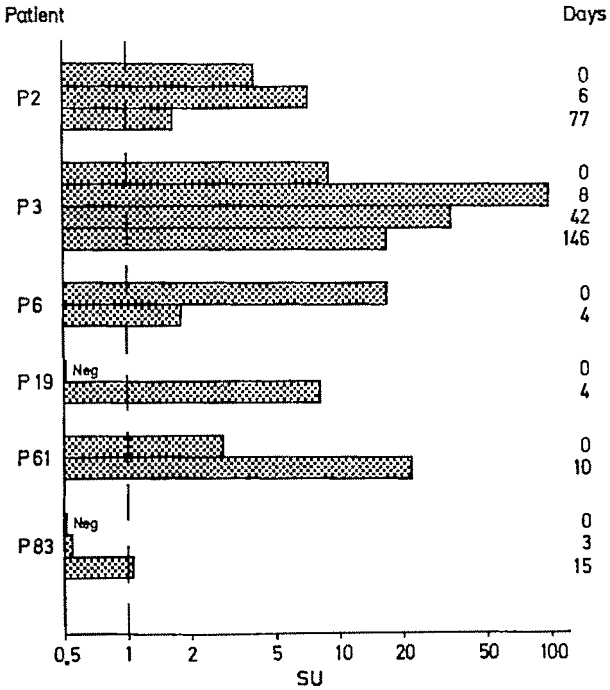

FIGURE 2. IgE-activity to benzylpenicilloyl-polylysine assayed by RAST in six patients' sera obtained at different time intervals after the first appearance of penicillin allergy symptoms. The results are expressed in sorbent units (SU).

reaction. Fourteen patients were observed up to 146 days after the first appearance of the clinical symptoms. Eleven patients had a positive RAST at the time of the allergic reaction, most of which became stronger later on and then decreased slowly after 10 days. Only three patients who had negative results at the beginning became positive after 4–15 days.

Serum IgE levels

Patients with acute reactions to penicillin (groups A and C) had significantly higher IgE levels ($P<0{\cdot}05$, mean 228 U/ml) than patients with a history of reactions to penicillin only (group B, 160 U/ml). When the first serum samples, taken at the beginning of the drug reactions, were compared to samples 1–6 days later (mean $3{\cdot}5\pm1{\cdot}5$) the levels had risen from 138·3 to 383·7 U/ml. The difference between these two values was highly significant ($P<0{\cdot}001$).

Passive haemagglutination

Titres higher than 1:80 could be found in seven patients belonging to groups A and C. Four had polymorphic exanthems, two anaphylactic shock and one urticaria. Six of these showed positive skin tests and/or RAST also. The seventh case, a patient with anaphylactic shock elicited by sodium penicillin G i.v., had only haemagglutinating antibodies (titre 1:80 on the first and 1:160 on the second day after the allergic reaction).

DISCUSSION

Many authors agree that reaginic antibodies, which were identified as IgE by Ishizaka, Ishizaka &

Hornbrook (1966), mediate the extraordinarily dangerous immediate type reactions in drug allergy (Halpern *et al.*, 1958; Layton, Yamamaka & Green, 1963; Redmond & Levine, 1967; Kraft *et al.*, 1972; de Weck, 1972; Girard, 1974). The performance of skin tests in such cases of suspected penicillin allergy was found to be useful for diagnosis, choice of antibiotic medication and prediction of possible complications in future penicillin treatment (de Weck, 1965; Levine & Zolov, 1969; Parker, 1970). But skin tests are time consuming, often not possible in patients with acute reactions and they bear a definite risk of generalized reactions, especially when performed with 'minor determinants'. A negative result does not exclude sensitization and positive results sometimes are observed in normal controls (Green, Rosenblum & Sweet, 1974).

In our investigations skin tests performed with 'major and minor determinants' were found to be positive in twenty-three (35·9%) of sixty-four patients with acute and former reactions to penicillin. PPL positive skin tests could be correlated with the BPO-specific RAST in 100% of cases which is an excellent result. But only three of eleven patients with positive skin tests to 'minor determinants' had cross-reacting IgE in the 'major determinant'—specific RAST and an *in vitro* diagnosis could not be made in the remaining eight patients. This fact suggests that RAST or other *in vitro* tests capable of detecting 'minor determinants'—specific IgE should be applied alongside the tests with 'major determinant' despite the fact that problems like instability of coupled antigens or undefined determinants limit their value for excluding penicillin allergy of the immediate type with certainty. Three well-documented cases with negative skin tests reacted positively in RAST and therefore the performance of RAST is to be recommended and can add further information to the skin tests.

In the present investigation the usual clinical manifestations in penicillin allergy were found (Idsoe *et al.*, 1968; Schulz, 1974). Positive RAST reactions were observed in patients with anaphylactic shock, urticaria and serum sickness-like reactions, but not in patients with scarlatiniform or morbilliform exanthems. From the latter diagnosis we found it important to differentiate other exanthematic eruptions, which are characterized by the existence of many different lesions appearing at the same time. These include large macular, often maculopapulovesicular and haemorrhagic lesions and recall occasionally erythema multiforme. In German publications these seem to be characterized best as 'between urticaria and morbilliform exanthems' (Steigleder, 1972) or by the term 'polymorph' (Nasemann & Sauerbrey, 1974); in English publications terms like 'exanthems', 'rashes' or 'exanthematic eruptions' are used (Levine & Zolov, 1969; Shapiro *et al.*, 1969; Baker, 1972). In our study the incidence of these exanthems was often connected with sodium penicillin G in high doses or with ampicillin treatment as previously observed in a few cases by Levine & Zolov (1969). In this group five of twenty patients showed specific IgE by means of RAST. Four of these had haemagglutinating antibodies in titres between 1:320 and 1:5120. In three cases delayed skin reactions could be observed after 48 h. These exanthems could not be clearly classified and represent, in our opinion, a complex response due to more than one immunological mechanism. Combinations of type I reactions with types III or IV according to Coombs & Gell (1975) may be of importance. Further information is necessary about specific antibodies of all the immunoglobulin classes that are present at the time of the allergic reaction. This could be obtained by the red cell linked antigen–antiglobulin reaction (Wheeler, 1971). Additionally, investigations of cell-mediated phenomena, e.g. by T-cell specific lymphocyte-transformation test, should be possible in the future and will throw further light on these allergic reactions. However, the role of IgE in the clinical picture must not be forgotten, as could be seen in one of these patients who gave evidence of incipient shock when skin tests were performed.

Specific IgE antibodies have been found in most cases of acute allergic reactions on the first day (twelve of fifteen patients). Therefore, a great advantage of RAST seems to be the possibility of making an early diagnosis in penicillin allergy. This is very important in intensive care units when a great number of drugs are given and penicillin has to be excluded as a reason for an allergic reaction. If the

RAST is negative on the day of the drug reaction, it should be repeated during the first weeks. Later a decrease of specific IgE in serum and skin is a limiting factor for both *in vitro* and *in vivo* tests and makes it sometimes impossible to find positive results months to years after the allergic reaction (Wide & Juhlin, 1971; Juhlin & Wide, 1972).

In patients with acute reactions to penicillin an early increase could be observed in the 'total' IgE levels, which corresponded only partly to the amounts of specific IgE antibodies. At the beginning of the reactions the patients showed significantly higher 'total' IgE levels than those with only a past history. Further observations displayed a decrease of 'total' IgE levels corresponding well with the fall in specific IgE antibodies as reported above.

The value of skin tests and RAST has been shown in this paper but it remains to be discovered what causes the drug side-effects in the remaining patients. Cell-mediated reactions have only been briefly mentioned. Similar skin lesions caused by non-allergic mechanisms must also be considered and it should also be pointed out that some patients had been treated with other drugs (dextrans, vitamins, etc.) besides penicillin when the drug reactions were observed.

REFERENCES

Baker, H. (1972) Drug reactions. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling) 2nd edn, Vol. 2, p. 1023. Blackwell Scientific Publications, Oxford.

Coombs, R.R.A. & Gell, P.G.H. (1975) Classification of allergic reactions responsible for clinical hypersensitivity and disease. In: *Clinical Aspects of Immunology* (Ed. by P.G.H.Gell, R.R.A.Coombs and P.J.Lachmann), 3rd edn, p. 761. Blackwell Scientific Publications, Oxford.

Girard, J.P. (1974) Diagnostic tests in drug allergy. In: *Allergology* (Ed. by Y. Yamamura *et al.*), p. 460. Excerpta Medica, Amsterdam.

Green, G.R., Rosenblum, A.H. & Sweet, L.C. (1974) Penicillin study group report. Summary of long term study. *Journal of Allergy*, **53**, 96 (Abstr.).

Halpern, B.N., Holtzer, A., Liacopoulos, P. & Meyer, J. (1958) Allergy to pyrazolone derivates (Amidopyrine) with evidence of a reaginic type antibody. *Journal of Allergy*, **29**, 1.

Idsoe, Ø., Guthe, T., Willcox, R.R. & de Weck, A.L. (1968) Nature and extent of penicillin side reactions, with particular reference to fatalities from anaphylactic shock. *Bulletin of the World Health Organisation*, **38**, 159.

Ishizaka, K., Ishizaka, T. & Hornbrook, M.M. (1966) Physicochemical properties of human reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. *Journal of Immunology*, **97**, 75.

Johansson, S.G.O., Bennich, H. & Wide, L. (1968) A new class of immunoglobulin in human serum. *Immunology*, **14**, 265.

Juhlin, L. & Wide, L. (1972) IgE antibodies and penicillin allergy. In: *Mechanisms in Drug Allergy* (Ed. by C.H. Dash & H.E.H.Jones), p. 139. Churchill Livingstone, Edinburgh and London.

Kraft, D., Werner, H.P., Stemberger, H. & Wiedermann, G. (1971) Der Einflusz von Penicillinase auf Hapteninhibitionen *in vitro* und *in vivo* bei experimenteller Penicillinallergie. *Zeitschrift für Immunitätsforschung, experimentelle und klinische Immunologie*, **141**, 265.

Kraft, D., Werner, H.P., Stemberger, H. & Wiedermann, G. (1972) Nachweis humaner Penicillinantikoerper vom IgE-Typ mittels Immunfluoreszenz und passiver kutaner Anaphylaxie in der Affenhaut. *Zeitschrift für Immunitätsforschung, experimentelle und klinische Immunologie*, **144**, 389.

Layton, L.L., Yamamaka, E. & Green, E. (1963) Atopic reagins to penicillin, pollens and seeds: thermolability, titer and persistence in the skin of passively sensitized macaqe monkeys. *International Archives of Allergy and Applied Immunology*, **31**, 87.

Levine, B.B., Fellner, M.J. & Levytska, V. (1966a) Benzylpenicilloyl-specific serum antibodies to penicillin in man. I. Development of a sensitive hemagglutination assay method and haptenic specificities of antibodies. *Journal of Immunology*, **96**, 707.

Levine, B.B., Fellner, M.J., Levytska, V., Franklin, E.C. & Alisberg, N. (1966b) Benzylpenicilloyl-specific serum antibodies to penicillin in man. II. Sensitivity of the hemagglutination assay method, molecular classes of the antibodies detected, and antibody titers of randomly selected patients. *Journal of Immunology*, **96**, 719.

Levine, B.B. & Zolov, D.M. (1969) Prediction of penicillin allergy by immunological tests. *Journal of Allergy*, **43**, 231.

NASEMANN, T.H. & SAUERBREY, W. (1974) *Lehrbuch der Hautkrankheiten und venerischen Infektionen*, p. 779. Springer, Berlin.

PARKER, C.W. (1970) Immediate skin reactions in penicillin hypersensitivity. In: *Penicillin Allergy* (Ed. by G.T. Stewart and J.P.McGovern), p. 81. Charles C.Thomas, Springfield.

REDMOND, A.P. & LEVINE, B.B. (1967) The relationship between immediate skin tests and passive transfer tests in man. *Journal of Allergy*, **39**, 51.

SCHNEIDER, C.H. (1974) Specific reactions to specific drugs: penicillin. In: *Allergology* (Ed. by Y.Yamamura *et al.*), p. 438. Excerpta Medica, Amsterdam.

SCHULZ, K.H. (1974) Arzneimittelallergische reaktionen der Haut; in Arzneimittelallergie. *Zeitschrift für Immunitätsforschung und experimentelle Therapie*, **1**, 177.

SHAPIRO, S., SISKIND, V., SLONE, D. & LEWIS, G.P. (1969) Drug rash with ampicillin and other penicillins. *Lancet*, **ii**, 969.

STEIGLEDER, G.K. (1972) *Dermatologie und Venerologie für Arzte und Studenten*, p. 191. G.Thieme, Stuttgart.

STEMBERGER, H., KRAFT, D., WERNER, H.P. & WIEDERMANN, G. (1971) Antigengemeinschaften zwischen Na-Penicillin G und Cephalosporin C-Derivaten. *Zeitschrift für Immunitätsforschung, experimentelle und klinische Immunologie*, **141**, 293.

STENIUS, B. & WIDE, L. (1969) Reaginic antibody (IgE), skin and provocation tests to Dermatophagoides culinae and house dust in respiratory allergy. *Lancet*, **ii**, 455.

STEWART, G.T. & MCGOVERN, J.P. (1970) *Penicillin Allergy*. Charles C.Thomas, Springfield.

VICKERS, M.R. & ASSEM, E.S.K. (1974) Tests for penicillin allergy in man. I. Carrier effect on response to penicilloyl conjugates. *Immunology*, **26**, 425.

DE WECK, A.L. (1965) Recent clinical and immunological aspects of penicillin allergy. *International Archives of Allergy and Applied Immunology*, **28**, 1113.

DE WECK, A.L. (1972) Critical evaluation of diagnostic methods in drug allergy. In: *Allergology* (Ed. by J.Charpin, C.Boutin, J.Aubert and A.W.Frankland), p. 23. Excerpta Medica, Amsterdam.

WHEELER, A.W. (1971) A method for measuring different classes of human immunoglobulins specific for the penicilloyl group. *Immunology*, **21**, 547.

WIDE, L. (1969) Radioimmunoassays employing immunosorbents. In: *Immunoassay of Gonadotrophins* (Ed. by E.Diczfalusy), *Acta endocrinologica*, Suppl. **142**, 207.

WIDE, L. (1973) Clinical significance of measurement of reaginic (IgE) antibody by RAST. *Clinical Allergy*, Suppl. **3**, 583.

WIDE, L., AXÉN, R. & PORATH, J. (1967) Radioimmunosorbent assay for proteins. Chemical couplings of antibodies to insoluble dextran. *Immunochemistry*, **4**, 381.

WIDE, L. BENNICH, H. & JOHANSSON, S.G.O. (1967) Diagnosis of allergy by an *in vitro* test for allergen antibodies. Lancet, **ii**, 1105.

WIDE, L. & JUHLIN, L. (1971) Detection of penicillin allergy of the immediate type by radioimmunoassay of reagins (IgE) to penicilloyl conjugates. *Clinical Allergy*, **1**, 171.

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DNA synthesis and mitosis in uninvolved epidermis of persistent palmoplantar pustulosis

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SUMMARY

Mitotic and DNA synthesizing cell counts have been performed in uninvolved epidermis of twenty-one patients with persistent palmoplantar pustulosis (PPP). There was no difference in mitotic counts and DNA synthesis in PPP compared with normal epidermis, but both were significantly lower than those found in the clinically uninvolved epidermis of patients with psoriasis.

Persistent palmoplantar pustulosis (PPP) is characterized by chronic sterile pustulation of the palms and soles. It is at present considered to be of unknown aetiology although its relationship to psoriasis is enigmatic. PPP is four times more common in patients with clinical psoriasis (Ingram, 1958) and is accompanied by psoriasis in 19–35% of patients (Everall, 1957; Ingram, 1958).

One of the characteristic features of psoriasis is the increase in the number of epidermal cells seen to be in mitosis and synthesizing DNA (Goodwin, Hamilton & Fry, 1973). Increase in mitosis (Goodwin *et al.*, 1973) and DNA synthesizing cells (Hell & Hodgson, 1966; Braun-Falco, Christophers & Kurban, 1967; Kaidbey & Kurban, 1971; Goodwin *et al.*, 1973) have also been observed in the clinically uninvolved epidermis of patients with psoriasis compared to normal epidermis. In order to elucidate further the relationship between PPP and psoriasis, mitosis and DNA synthesis have been studied in PPP.

PATIENTS AND METHODS

Forearm skin of twenty-one patients with PPP was studied. They were aged 45–65 years and the duration of their disorder ranged from 4 months to 18 years. Four of the patients had minimal psoriasis, in three on the elbows only. One of these and three others were being treated with oral triamcinolone. No patient had arthritis.

A 4 mm punch biopsy was performed following 2% lignocaine local anaesthesia. 10 μc of ^{3}H-thymidine had been injected intra-epidermally into the site 45 min previously. The specimens were fixed in formol saline, embedded in wax and sections 5 μm thick were cut. Mitotic counts were observed from sections stained with haematoxylin and eosin. Labelled DNA synthesizing cells were counted from autoradiographs prepared by coating sections with Kodak AR10 stripping film and incubating for 3 weeks at 4°C. In all specimens 100 high power fields (× 400) were counted, as described by Fry & McMinn (1966).

Mitotic counts and labelled cell counts were compared with those similarly obtained in a previous study of the clinically uninvolved epidermis of psoriasis and normal epidermis (Goodwin *et al.*, 1973) (Table 1).

RESULTS

The mean mitotic count in the twenty-one specimens was 0·30 with a range of 0–1. The mean number of labelled cells was 182 (±18) with a range of 120–238 (Table 1).

TABLE 1. Comparison between mean mitotic counts and mean number of DNA synthesizing cells in the epidermis

	Uninvolved psoriasis	Uninvolved PPP	Normal
Mitotic count	1·9±0·5	0·30	0·33
Labelled cell count	362±32	182±18	150±19

There is a significant difference between both mitotic counts and DNA synthesizing cells in uninvolved epidermis in PPP and psoriasis ($P < 0·001$). There is no significant difference between DNA synthesizing cells in uninvolved epidermis in PPP and normal epidermis ($P > 0·10$).

Although the numbers are small there appeared to be no difference in the mitotic counts and labelled cell counts between the four patients with PPP who also had mild clinical psoriasis and the other seventeen patients who did not have psoriasis. Likewise there was no difference in the four patients taking triamcinolone compared to those not taking this drug.

DISCUSSION

These studies show that judged by mitosis and DNA synthesizing cells the uninvolved epidermis in PPP is similar to normal epidermis and does not show the increases seen in the clinically uninvolved epidermis of psoriasis (Hell & Hodgson, 1966; Braun Falco *et al.*, 1967; Kaidby & Kurban, 1971; Goodwin *et al.*, 1973). Thus it would seem that PPP is not a variant of psoriasis but is a separate entity. This suggestion is supported by the fact that finger-nail growth is not increased in PPP as in psoriasis (Dawber & Byrne, 1975). HL-A antigen studies (Svejgaard *et al.*, 1974) have also shown a difference between patients with PPP and those with psoriasis. In the latter there is an increased incidence of HL-A 13 and 17.

Oral and topical steroids are known to be able to inhibit mitosis and DNA synthesis in human epidermis. However, in the four patients in this study who were taking triamcinolone there was no apparent decrease in DNA synthesis, compared to patients not taking this drug. Thus the therapeutic effect of this drug in PPP is unlikely to be due to its effect on DNA synthesis.

It might have been expected that the mitotic and DNA synthesizing cell counts would be increased in our patients with psoriasis. These had minimal psoriasis usually only of the elbows. Studies relating mitotic and DNA synthesizing activity in uninvolved psoriatic epidermis to the extent of the psoriasis have not been performed. It is possible that when the psoriasis is relatively quiescent the cellular activity in uninvolved epidermis also tends to be normal. One patient with PPP and extensive psoriasis, who has been studied subsequently, had a DNA synthesizing cell count of 447, and it is possible that DNA synthesis in uninvolved psoriasis is related to the general activity of the disease.

At present there would appear to be little evidence for linking PPP to psoriasis and considering it to be a variant of the disease. Further investigations are required into the nature of both disorders before making any speculative claims as has been done in the past.

TABLE 2

Patients	Age	Sex	Duration of disease	Mitotic counts	DNA count (per 100 high power fields × 400)
1†	50	M	17/12	0	180
2	62	M	2 yrs	1	168
3	65	F	3 yrs	1	138
4	45	M	2 yrs	0	184
5†*	48	M	9/12	0	196
6	48	M	1 yr	0	236
7	52	M	16/12	0	170
8	49	M	3 yrs	0	180
9†	47	F	5 yrs	0	190
10	48	M	3 yrs	0	208
11	60	M	7 yrs	0	160
12	58	F	7/12	0	166
13*	59	F	18 yrs	0	166
14†	63	F	12 yrs	0	120
15	57	M	4 yrs	0	220
16	56	M	8 yrs	0	150
17*	49	F	9/12	0	166
18	60	F	3 yrs	0	224
19	49	M	3 yrs	0	238
20*	58	F	7/12	0	174
21	52	F	4/12	0	155

* Patients also had psoriasis.

† Patients taking oral triamcinolone.

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REFERENCES

BRAUN-FALCO, O., CHRISTOPHERS, T. & KURBAN, A. (1967) Abnormes Verhalten der Epidermalen Regeneration bei Patienten mit Psoriasis Vulgaris. *Archiv für klinische und experimentelle Dermatologie*, **229**, 276.

DAWBER, R.P.R. & BYRNE, J.P.N. (1975) Finger nail growth in peripheral pustulosis. *British Journal of Dermatology*, **93**, Suppl. 11, 24.

EVERALL, J.D. (1957) Intractable pustular eruptions of the hands and feet. *British Journal of Dermatology*, **69**, 269.

FRY, I. & MCMINN, R.M.H. (1966) Action of methotrexate on skin and intestinal epithelium in psoriasis. *Archives of Dermatology*, **93**, 726.

Goodwin, P., Hamilton, S. & Fry, L. (1973) A comparison between DNA synthesis and mitosis in uninvolved and involved psoriatic epidermis. *British Journal of Dermatology*, **89**, 613.

Hell, E. & Hodgson, C. (1966) The uptake of ^{3}H-thymidine by epidermal cells in normal and psoriatic subjects. *British Journal of Dermatology*, **78**, 262.

Ingram, J.T. (1958) Pustular psoriasis. *Archives of Dermatology*, **77**, 314.

Kaidbey, K.H. & Kurban, A.K. (1971) Mitotic behaviour of the buccal mucosal epithelium in psoriasis. *British Journal of Dermatology*, **85**, 162.

Svejgaard, A., Neilsen, L.S., Svejgaadt, E., Neilsen, F.K. & Hjortshoj, A. (1974) HL-A in psoriasis vulgaris and in pustular psoriasis—population and family studies. *British Journal of Dermatology*, **91**, 145.

British Journal of Dermatology (1976) **94**, 607.

Glucose-6-phosphate dehydrogenase in lichen planus skin

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SUMMARY

The apparent Michaelis constant (Km) for glucose-6-phosphate of the enzyme glucose-6-phosphate dehydrogenase has been measured in extracts prepared from biopsies of normal human skin and from both affected and apparently normal skin of patients with lichen planus. No differences of Km were found and starch gel electrophoresis of extracts from lichen planus lesions and normal controls showed similar patterns when stained for glucose-6-phosphate dehydrogenase activity. These results do not support the view that lichen planus is an inborn error of metabolism in which the structure of glucose-6-phosphate dehydrogenase of skin is affected.

Cotton, Van den Hurk & Van der Staak (1972) reported that the Km of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) for glucose-6-phosphate was increased in extracts prepared from biopsies obtained from the skin of patients with lichen planus. This result was found both when the biopsy was obtained from a lesion and from apparently unaffected skin, and was independent of age, sex or site. In consequence they suggested that glucose-6-phosphate dehydrogenase in the skin of patients with lichen planus might be structurally abnormal and that the disease may be an inborn error of metabolism.

Although an alteration in Km may indicate that an enzyme differs structurally from normal (and hence is specified by a mutant gene) other explanations are possible. In the first place, isoenzymes of the enzyme are known (Ohkawara *et al.*, 1967), and differences in Km may reflect differences in isoenzyme composition. Secondly, allosteric inhibitors commonly increase the Km of an enzyme for its substrate and it is possible that extracts of lichen planus skin contain an inhibitor of this kind. A third possibility derives from the fact that, in the experiments of Cotton *et al.*, the activity of glucose-6-phosphate dehydrogenase was determined by measuring the rate of formation of NADPH as shown in the following equation:

$$\text{Glucose-6-phosphate} + \text{NADP}^+ \rightarrow 6\ \text{phosphogluconate} + \text{NADPH} + \text{H}^+$$

However, skin extracts also contain 6-phosphogluconate dehydrogenase (EC 1.1.1.44) which catalyses the reaction:

$$\text{6-phosphogluconate} + \text{NADP}^+ \rightarrow \text{ribulose-5-phosphate} + \text{NADPH} + \text{H}^+ + \text{CO}_2$$

Thus the presence of 6-phosphogluconate dehydrogenase may complicate the assay of glucose-6-phosphate dehydrogenase by this method (Glock & McLean, 1953), and in general the effect is that the apparent Km is lower than the true value.

In view of these uncertainties, we decided to measure some of the kinetic properties of glucose-6-phosphate dehydrogenase in skin from patients with lichen planus and from controls.

METHODS

Patients with lichen planus showed clinical and/or histological evidence of this condition. Biopsies were taken from lichen planus lesions and from the apparently unaffected skin of these patients, and from the normal thigh skin of patients attending the clinic for removal of warts and naevi. Xylocaine was used as local anaesthetic and biopsies were transferred to sterile ice-cold isotonic saline.

Subcutaneous fat was removed and the weighed samples (20–40 mg) were homogenized in 1·0 ml 0·15 M-sodium chloride for 1 min at 0–4°C using an all glass Potter type homogenizer with a gap of 0·01 mm. The extract was centrifuged at 16000 *g* for 5 min at 0–4°C and the clear supernatant used for enzyme assay, and for the estimation of soluble protein (Lowry *et al.*, 1951).

Assay of glucose-6-phosphate dehydrogenase

The assays were carried out in silica cuvettes in an Aminco-Bowman spectro-fluorimeter at 37°C with excitation wavelength of 340 nm and emission wavelength 460 nm. The reaction medium was 0·1 M-tris buffer pH 7·3 containing 3 mM-$MgCl_2$, 0·5 mM-EDTA, 0·05 mM-NADP and concentrations of glucose-6-phosphate varying between 0·05 and 1 mM. The reaction was started by the addition of skin extract and was shown to be linear with time. A minimum of three concentrations of glucose-6-phosphate was used and the fluorescence produced during 15 min incubation at 37°C due to the formation of NADPH was measured. The Km for glucose-6-phosphate was determined from a Lineweaver–Burk plot of the reciprocals of glucose-6-phosphate concentration and initial reaction velocity.

Assay of glucose-6-phosphate dehydrogenase in the presence of excess 6-phosphogluconate dehydrogenase

To the reaction mixture was added 1·75 units of 6-phosphogluconate dehydrogenase (Boehringer). The rates of NADPH production were divided by 2, and the values obtained were used in the calculation of Km of glucose-6-phosphate dehydrogenase for glucose-6-phosphate.

Starch gel electrophoresis

Vertical starch gel electrophoresis and tetrazolium staining of the glucose-6-phosphate dehydrogenase activity were carried out under the conditions given in the W.H.O. report on the standardization of procedures for the study of glucose-6-phosphate dehydrogenase (1967).

RESULTS

Using the assay for glucose-6-phosphate dehydrogenase, without any added 6-phosphogluconate dehydrogenase, it can be seen in Fig. 1 that the Lineweaver–Burk plot for glucose-6-phosphate is linear, and the value of Km is unaffected by varying the enzyme concentration over a five-fold range.

The Km values calculated for the three groups of samples, namely, normal controls, lichen planus lesions and biopsies from clinically unaffected areas of patients with lichen planus, are given in Fig. 2.

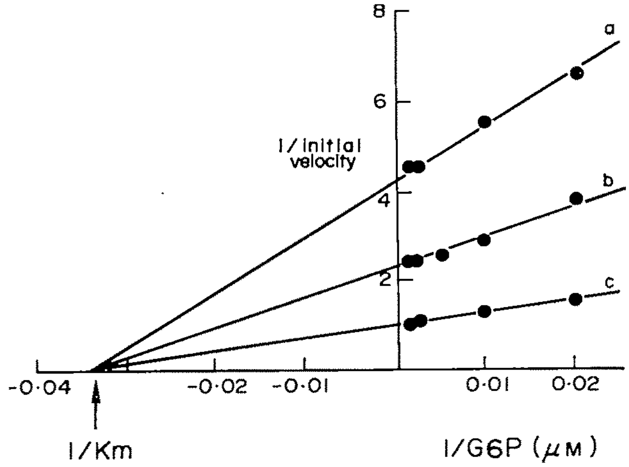

FIGURE 1. Lineweaver-Burk plot for skin glucose-6-phosphate dehydrogenase at three enzyme concentrations. (a) 3 μg protein; (b) 6 μg protein; (c) 15 μg protein.

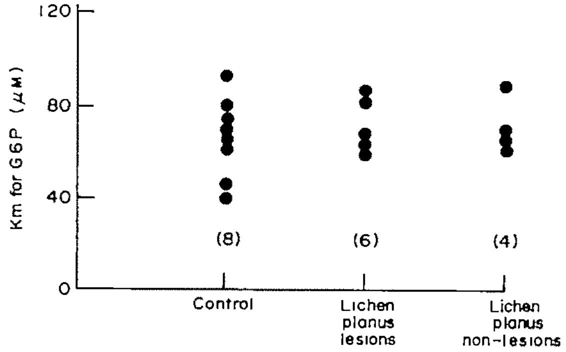

FIGURE 2. Km values for glucose-6-phosphate in normal control skin, lichen planus lesions and unaffected skin in patients with lichen planus. Number of observations in parentheses.

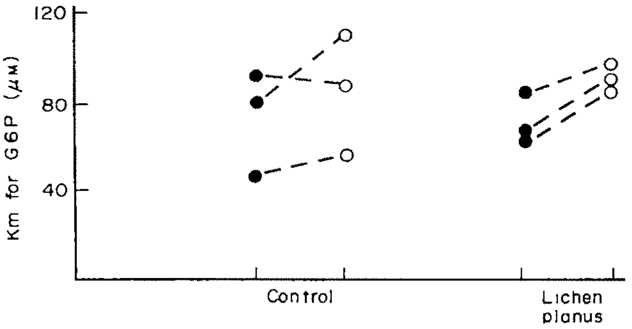

FIGURE 3. The effect of added excess 6-phosphogluconate dehydrogenase upon observed glucose-6-phosphate dehydrogenase activity.

They show that extracts prepared from lichen planus patients, either from lesions or from unaffected skin, are within the range found for the normal controls.

The effect of including 6-phosphogluconate dehydrogenase in the assay medium on the Km of glucose-6-phosphate dehydrogenase for glucose-6-phosphate is shown in Fig. 3. As expected the Km is increased but the effect is small and of similar magnitude in extracts from lichen planus lesions and from normal skin.

Starch gel electrophoresis of extracts from lichen planus lesions and from normal control skin showed two bands of activity due to glucose-6-phosphate dehydrogenase. However, the bands seen in the extracts prepared from lichen planus lesions were indistinguishable in both position and intensity from those observed in extracts of control skin.

DISCUSSION

The values reported above for the apparent Km of glucose-6-phosphate dehydrogenase of normal skin agree well with the values of 50–116 μM glucose-6-phosphate found by Cotton *et al.* (1972), but we are unable to confirm their finding that in patients with lichen planus there is an increased Km for glucose-6-phosphate dehydrogenase extracted from biopsies of either lesions or apparently unaffected skin.

Our experiments show that the determination of Km is not influenced to any important extent by the presence of 6-phosphogluconate dehydrogenase in the skin extracts and no evidence for an allosteric inhibitor has been obtained. In consequence, our findings do not support the view that in lichen planus glucose-6-phosphate dehydrogenase in skin is structurally abnormal, and this conclusion is strengthened by the observation that on starch gel electrophoresis no differences were observed.

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REFERENCES

COTTON, D.W.K., VAN DEN HURK, J.J.M.A. & VAN DER STAAK, W.B.J.M. (1972) Lichen planus: an inborn error of metabolism. *British Journal of Dermatology*, **87**, 341.

GLOCK, G.E. & MCLEAN, P. (1953) Further studies on the properties and assay of glucose-6-phosphate dehydrogenase of rat liver. *The Biochemical Journal*, **55**, 400.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265.

OHKAWARA, A., HALPRIN, J., BARBER, P. & HALPRIN, K.M. (1967) Human epidermal isoenzymes. *Archives of Dermatology*, **95**, 412.

Standardization of Procedures for the Study of Glucose-6-phosphate dehydrogenase. World Health Organisation Technical Reprint Service (1967), **366**.

British Journal of Dermatology (1976) **94**, 611.

Effects of short chain alcohols and hydrocarbon compounds on the adenylate cyclase of the skin

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SUMMARY

The cyclic AMP content of epidermal slices is increased by incubation with ethanol, the effect of which is dose-dependent from 1 to 5% concentration in the incubation media.

n-Propanol and acetone are also effective at a concentration equimolar to 5% ethanol.

Experimental results suggest that this effect of ethanol is due to activation of adenylate cyclase, rather than to the inactivation of cyclic AMP-phosphodiesterase.

During studies on the effect of prostaglandins on epidermal adenylate cyclase, we observed significant elevations of the cAMP (adenosine 3′,5′-monophosphate) content of specimens incubated with ethanol alone as a solvent control. Although it has been reported that some short chain alcohols can stimulate adenylate cyclase activity in the broken cell preparations (Gorman & Bitensky, 1970; Satoh & Ryan, 1972; Kuo, Hodgins & Kuo, 1973; Mashiter, Mashiter & Field, 1974), no such effect has been reported with an intact tissue as a sample. The following studies were carried out to see the effect of ethanol and some related compounds on the adenylate cyclase in unbroken epidermal slices.

MATERIALS AND METHODS

A sheet of skin was removed from the dorsum of a young white pig by a Castroviejo keratome set at 0·3 mm thickness, and kept in Hanks's balanced salt solution. The tissue was used for experiments within 2 h after removal from the body. The tissue was cut into pieces approximately 0·7 × 0·7 cm, floated epidermal side up on Hanks's balanced salt solution and pre-incubated at 37°C for 15–20 min. After the pre-incubation, two pieces of skin were transferred to 3 ml of incubation medium, composed of Hanks's balanced salt solution, 10 mM theophylline and various test agents, and incubated at 37°C for 5 min. After this incubation, the tissues were quickly frozen between flat pieces of dry ice, and analysed for cAMP content by the modified protein binding method of Gilman (1970). Details of these procedures are the same as previously reported (Yoshikawa *et al.*, 1975a,b). Tissue protein was measured by the method of Lowry *et al.* (1951).

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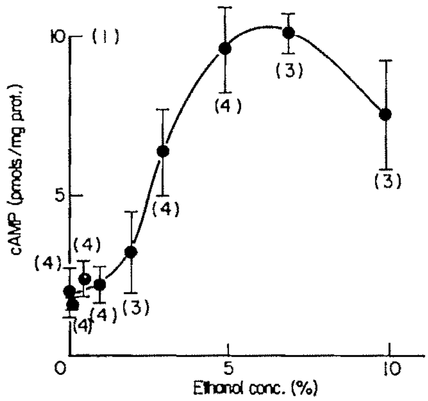
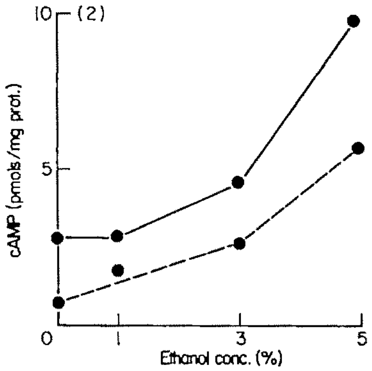
RESULTS

Effects of ethanol concentration

Results are shown in Fig. 1. Cyclic AMP increased as the ethanol concentration in the incubation medium was increased to 5%, reached a maximum at about 6%, then decreased when the ethanol concentration was further increased. No remarkable effects were noted at ethanol concentrations below 1%. Fig. 2 demonstrates the effect of ethanol at various concentrations in the presence and absence of 10 mM theophylline. The increase in cAMP in relation to increased ethanol concentration





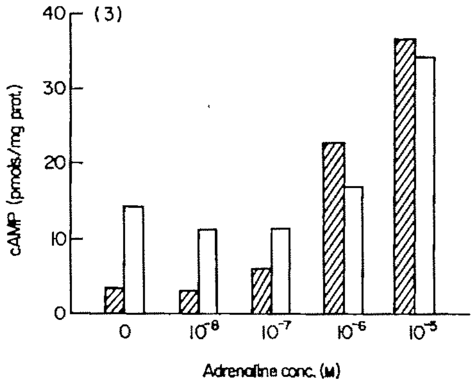


FIGURE 1. Cyclic AMP accumulation in the epidermal slices incubated with ethanol of various concentrations. Results are shown as average ± s.e. mean. Number of experiments is shown in parentheses.

FIGURE 2. Additive effect of theophylline on ethanol-induced cAMP accumulation in the epidermis. Tissues are incubated with various concentrations of ethanol in the presence of (———) and absence of (– – – – –) 10 mM theophylline.

FIGURE 3. Effect of 5% ethanol on the adrenaline stimulation of epidermal adenylate cyclase. Tissues are incubated with (open columns) or without (hatched columns) 5% ethanol in addition to various concentrations of adrenaline.

is noted in both groups. Tissues with theophylline always showed a greater cAMP concentration. Since inhibition of cyclic AMP-dependent phosphodiesterase alone by theophylline did not produce a large increase in cAMP in this experimental system (Yoshikawa *et al.*, 1975a,b), it is concluded from these results that this ethanol effect is due to stimulation of cAMP formation rather than to suppression of cAMP degradation.

Digestion of tissue extracts by exogenous phosphodiesterase completely eliminated the measured cAMP accumulated by ethanol, as well as that by adrenaline, thus confirming that the substance measured actually was cAMP.

When the tissue was heated at 100°C for 2 min prior to incubation, this effect of ethanol was not observed.

These data suggest that ethanol increases the cAMP content of epidermis by means of activation of adenylate cyclase.

Effect of various alcohols and related compounds

Methanol, n-propanol, n-butanol, glycerol, acetone, and acetate were tested for their effects on adenylate cyclase activity at equimolar concentrations to 5% (0·87 M) ethanol.

At this molar concentration, n-propanol and acetone were as effective as ethanol, while acetate showed slight stimulation and the rest were ineffective. The results are summarized in Table 1.

TABLE 1. Effects of some alcohols and hydrocarbon compounds. Concentration of all test compounds is 0·87 M, which is equimolar to 5% ethanol. Acetate is added to the media as sodium acetate, and shift of pH is compensated for with the use of CO_2

Additives (0·87 M)	cAMP (pmol/mg prot.)		
	Experiment 1	Experiment 2	Average
None	1·38	3·62	2·50
Methanol	2·22	3·30	2·76
Ethanol	11·07	14·35	12·71
n-Propanol	8·95	13·96	11·46
n-Butanol	2·11	3·55	2·83
Glycerol	3·16	1·94	2·55
Acetone	14·68	12·20	13·44
Acetate	4·32	6·28	5·30

Effect of adrenaline on ethanol-stimulated adenylate cyclase

To see whether ethanol would have any effect on other sites where adenylate cyclase stimulation occurs, incubation was carried out in the presence of various concentrations of adrenaline, a potent known stimulator (Yoshikawa *et al.*, 1975a,b), in addition to the 5% ethanol.

As shown in Fig. 3, ethanol showed a definite effect at low adrenaline concentrations where adrenaline did not show notable stimulatory effects.

When the adrenaline concentration was increased ($10^{-6} \sim 10^{-5}$ M), the effect of ethanol was completely masked, and when both agents were put together they did not show any additive effect even at adrenaline concentrations that were submaximal.

DISCUSSION

The effect of short chain alcohols (methanol to butanol) on the glucagon-sensitive adenylate cyclase in broken liver cell membranes has been extensively studied by Gorman & Bitensky (1970). According to their observations, all these alcohols and their isomers were effective, with optimal concentrations inversely related to their molecular weights. The effect was specific for the sensitive enzyme and was produced by changing Vmax and Km of the enzyme reversibly. The stimulatory effect of ethanol on the broken cell enzyme of placenta (Satoh & Ryan, 1972), pancreatic islet cells (Kuo *et al.*, 1973) and thyroid (Mashiter *et al.*, 1974) has also been reported.

Although no such effect of 1% ethanol was observed using tissue slices of thyroid (Mashiter *et al.*, 1974), our experiments clearly showed an effect on intact epidermis. The optimal concentration of ethanol (about 6%) was very close to that (5%) reported on the broken cell preparation of the liver (Gorman & Bitensky, 1970). n-Propanol and acetone were found to be as effective as ethanol in our study. No such effects by acetone have been reported previously. It widens the specificity beyond the alcohols.

Gorman & Bitensky (1970) observed that this effect was seen only on the glucagon-sensitive but not on the adrenaline-sensitive enzyme of the liver with the changes in Km and Vmax. Therefore, they proposed a rather specific effect of alcohols by changing the environment of direct binding to the enzyme. In the case of the skin, adrenaline is a potent activator of adenylate cyclase, while preliminary studies do not show any effect of glucagon on the epidermal enzyme. Neither additive stimulation nor enhancement of the adrenaline stimulation was seen. In the presence of a strong specific activator (adrenaline), the alcohol effect cannot be seen. It seems likely from this result therefore, that ethanol is acting non-specifically on the cell membrane to activate adenylate cyclase in the epidermis. Detailed study is required to fully understand this phenomenon.

Because of the high concentration of the agents required to give their effects, it is hard to believe this effect has any physiological role *in vivo*, although some of their pharmacological or toxic effects could be understood through this phenomenon. These effects should also be kept in mind whenever these compounds are used as solvent or additives in experiments on the cAMP system.

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REFERENCES

GILMAN, A.G. (1970) A protein binding assay for adenosine 3′,5′-cyclic monophosphate. *Proceedings of the National Academy of Sciences of the United States of America*, **67**, 305.

GORMAN, R.E. & BITENSKY, M.W. (1970) Selective activation by short chain alcohols of glucagon responsive adenyl cyclase in liver. *Endocrinology*, **87**, 1075.

KUO, U.N., HODGINS, D.S. & KUO, J.F. (1973) Adenylate cyclase in islets of Langerhans. *Journal of Biological Chemistry*, **248**, 2705.

LOWRY, O.H., ROSENBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265.

MASHITER, K., MASHITER, G.D. & FIELD, J.B. (1974) Effects of prostaglandin E_1, ethanol and TSH on the adenylate cyclase activity of beef thyroid plasma membranes and cyclic AMP content of dog thyroid slices. *Endocrinology*, **94**, 370.

SATOH, K. & RYAN, K.J. (1972) Prostaglandins and their effects on human placental adenyl cyclase. *Journal of Clinical Investigation*, **51**, 456.

YOSHIKAWA, K., ADACHI, K., HALPRIN, K.M. & LEVINE, V. (1975a) Cyclic AMP in skin: effects of acute ischemia. *British Journal of Dermatology*, **92**, 249.

YOSHIKAWA, K., ADACHI, K., HALPRIN, K.M. & LEVINE, V. (1975b) The effects of catecholamine and related compounds on the adenyl cyclase system in the epidermis. *British Journal of Dermatology*, **93**, 29.

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The significance of the local sweat response in assessing the progress of leprosy

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SUMMARY

Serial observations on cutaneous sensation and on sweat response in hypopigmented flat patches of twenty-nine patients with tuberculoid and five with dimorphous leprosy on dapsone therapy were studied over 2 years. Sensation was determined by routine methods, while sweat stimulation was done by intradermal injection of 0·1 ml (0·025 mg) of carbachol (carbaminoylcholine). There was significant improvement in sensation and considerable augmentation of sweat response in the final as compared to the initial tests. The difference in sweat response was statistically significant, suggesting the value of this simple procedure in assessing the progress of leprosy.

It is now well recognized that *Mycobacterium leprae* inflicts insidious damage on both autonomic and somatic nerve fibres, resulting in impairment or loss of temperature, touch and pain sensation in the hypopigmented leprous patches (Lumsden, 1964). Likewise sweating is also impaired in these patches, causing partial or complete anhidrosis. These pathological changes are certainly reversible if specific therapy with dapsone (diaminodiphenyl sulphone) is instituted early. Qualitative assessment of recovery of cutaneous sensation in such patients can be observed by routine procedures along with the subjective recovery of sweating in hypopigmented leprous patches. The significance of the local sweat response in the diagnosis of leprosy has been illustrated recently (Sehgal, 1974), and in a few earlier reports (Arnold, 1948; Parikh *et al.*, 1966; Mathur *et al.*, 1971). It was therefore decided to study sensation and sweat response serially for a period of 2 years in leprosy patients on dapsone treatment to determine the value of sweat testing in assessing the progress of the disease.

MATERIALS AND METHODS

Thirty-four patients with tuberculoid or with dimorphous leprosy were selected. Their age ranged from 13 to 60 years. The diagnosis was made on clinical symptoms, signs and histopathological examination. The sensations of temperature, touch and pain were carefully determined by routine methods. The patients were classified into five groups according to their sensory status.

0·1 ml (0·025 mg) of carbachol was injected intradermally into hypopigmented flat patches and the

Request reprints: Professor V.N. Sehgal, MD., M.A.M.S. (as above).

area was left covered with Whatman No. 1 filter paper strip impregnated with 1% alcoholic solution of bromophenol blue. The paper was removed after 5 min and the number of blue dots indicative of sweating was counted for an area 2·5 cm^2 around the site of injection. The central smudge at the site of injection was discounted. The results were expressed as: Nil (0–10 spots); + (11–100 spots); + + (101–200 spots); + + + (201–300 spots); + + + + (more than 300 spots or large smudges of sweat). Contralateral normal sites were used as controls.

Each patient received orally dapsone 6·0–10 mg per kg/body weight per week in equal divided dosages daily for a period of 2 years.

The serial tests of sensation and sweating were done at regular intervals of 3 months for 2 years. The final results were compared with the initial ones, both being done in corresponding months of the year, thus eliminating the possible influence of variations in environmental temperature and humidity.

OBSERVATIONS

There were twenty-nine patients (13 male) with tuberculoid and five (all male) with dimorphous leprosy. The distribution of the patients according to the sensory changes in the leprosy lesions before (Initial) and after 2 years treatment (Final) in the different groups is shown in Table 1.

TABLE 1. Sensory status of the lesions, initial and final

Groups	Sensory status: temperature, touch and pain	Initial no. of patients	Final no. of patients					
			1	2	3	4	5	Others
1	All 3 normal	1	1	—	—	—	—	—
2	All 3 impaired	9	1	7	—	—	—	1
3	2 impaired 1 lost	3	—	2	—	—	—	1
4	1 impaired 2 lost	4	—	3	1	—	—	—
5	All 3 lost	17	1	11	1	—	2	2
Total		34	3	23	2	—	2	4

It can be seen from Table 1 that initially there were seventeen patients who had complete loss of temperature, touch and pain sensation. In one patient all sensations were normal. Nine patients had impairment of all the three sensations. In three patients two of the sensations were impaired and the other was lost, while in the remaining four patients one of the sensations was impaired and two were lost.

The recovery of the sensations of temperature, touch and pain is apparent from the same table. Of the seventeen patients in group 5 all three sensations had completely reverted to normal in one while in eleven others there was only partial recovery. In two patients touch and pain had returned to normal but the temperature sensation showed only partial improvement. Of the remaining three patients, one showed improvement in touch and pain while in the other two patients the sensations remained unchanged. Of the nine patients in group 2, one patient had regained all three sensations whilst another showed normal touch and pain sensations. A similar tendency was seen in groups 3 and 4 with the return of normal pain sensation in one of them.

TABLE 2. Correlation of cutaneous sensations and sweat response

Group	Sensory status: temperature, touch and pain	No. of cases	Sweat response Initial Mean	Initial SD*	Final Mean	Final SD	'*t*'	Significance (*P* value)
1	All 3 normal	1	0·00	—	2·00	—	—	—
2	All 3 impaired	9	1·00	±0·82	2·55	±0·68	4·603	0·005
3	2 impaired 1 lost	3	0·00	—	—	—	—	—
4	1 impaired 2 lost	4	1·25	—	1·75	—	—	—
5	All 3 lost	17	0·71	±0·53	2·17	±0·51	11·655	0·001

* Standard deviation not computed when the number studied was small.

The mean and standard deviation for the initial and the final sweat response for each group are shown in Table 2. The mean initial sweat response in seventeen patients who had loss of all three sensations (group 5) was 0·71 in contrast to 2·17 in the final, after completion of 2 years treatment. The difference in the two values is highly significant ($P<0{\cdot}001$). In nine leprosy patients whose sensations of temperature, touch and pain were impaired (group 2) the mean sweat responses in the initial and final tests were 1·00 and 2·55 respectively, the difference being significant ($P<0{\cdot}005$). It was not possible to compute standard deviations and tests of significance in groups 1, 2 and 4 as the numbers studied were too small.

The comparison between groups 2 and 5 in respect of initial and final sweat response is shown in Table 3. No significant difference in values of sweat response was noted between these groups. The degree of repigmentation of hypopigmented flat patches could not be correlated with sweat response.

DISCUSSION

The improvements in sensation and in the sweat response were noted concurrently in the present study. The value of testing sensation in the follow-up of leprosy patients is well known. The value of serial local sweat tests in assessing the progress of tuberculoid and dimorphous leprosy on dapsone therapy is clearly demonstrated in the present communication. It is, however, imperative to establish the diagnosis of leprosy early to ensure favourable therapeutic results. The clinical response to dapsone is apparent within a short time, but complete regression of the skin and the nerve lesions takes much longer and is variable. Furthermore, adequate dosage of dapsone facilitates the regeneration of the somatic and autonomic nerves, resulting in recovery of sensations of temperature, touch and pain and also of sweating. Sensory testing is a simple clinical procedure, but is subjective and

TABLE 3. Test of significance between groups

Groups	'*t*'	Inference	*P* value
Initial 2 vs 5	1·055	n.s.	0·05
Final 2 vs 5	1·544	n.s.	0·05

n.s. = not significant.

qualitative. The local sweat test is objective and quantitative. Thus it is beneficial to use the local sweat test in addition to sensory tests to evaluate the prognosis in leprosy. It is cheap and convenient to execute.

ACKNOWLEDGMENTS

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REFERENCES

Arnold, N.L. (1948) The intradermal mecholyl test for anhidrosis, a diagnostic aid in leprosy. *International Journal of Leprosy*, **16**, 335.

Lumsden, C.E. (1964) Leprosy and the Schwann cells *in vivo* and *in vitro*. *Leprosy in theory and practice* (Ed. by R.G.Cochrane and T.F.Davy), pp. 221–250. Wright, Bristol.

Mathur, N.K., Pasricha, J.S., Dharam Pal & Singh, N. (1971) Comparison of the cutaneous autonomic and somatic nervous functions in lesions of leprosy. *International Journal of Leprosy*, **39**, 146.

Parikh, A.C., Ganapati, R., Kapadia, B.I. & Naik, S.S. (1966) Acetylcholine test for anhidrosis in leprosy. *Leprosy Review*, 37, 231.

Sehgal, V.N. (1974) Significance of the local sweat response in the diagnosis of leprosy. *Dermatologica*, **148**, 217.

British Journal of Dermatology (1976) **94**, 619.

Hypersensitivity to bacteria in eczema

I. BACTERIAL CULTURE, SKIN TESTS AND IMMUNOFLUORESCENT DETECTION OF IMMUNOGLOBULINS AND BACTERIAL ANTIGENS

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SUMMARY

A study was made of the cytotoxic effect of antibacterial antibody and complement reacting with bacterial antigens firmly adsorbed to epidermal cells. It is believed that this phenomenon enhances the severity of the lesions and their spread in some cases of disseminated eczema.

In this first part of the study it is confirmed that *Staphylococcus aureus* and micrococci are frequently present on lesions and 'unaffected' skin of patients with disseminated eczema. Intradermal skin tests with antigens of staphylococci and micrococci on 122 eczematous patients elicited immediate, or combined immediate and 4 h (Arthus-like) responses, in a large proportion, but few showed uncombined 4 h responses or delayed hypersensitivity, in contrast to findings reported by others.

Immunofluorescence tests on skin of thirty patients showed that IgG and IgM diffused into the epidermis, sometimes to the skin surface, of lesional skin, and more immunoglobulin was found in skin of 'unaffected' areas than in skin of normal healthy persons, indicating that clinically unaffected skin in patients with disseminated eczema is abnormal. IgD was present in three of eight samples of unfixed, and six of eight samples of fixed eczematous skin. Staphylococcal and micrococcal antigen was shown on the skin surface and also diffusely in the cytoplasm of cells in the dermis beneath the surface deposits, indicating percutaneous absorption. Further small amounts of antigen were adsorbed to some epidermal cells.

These results show that the predisposing conditions for a cytotoxic reaction mediated by hypersensitivity to bacteria do occur. Increased growth of staphylococci and micrococci on eczematous skin would result in increased deposits of antigen. Bacterial antigens are absorbed into the skin and bind with epidermal cells, and immunoglobulins diffuse into the epidermis. Furthermore, skin tests showed that many eczematous patients were hypersensitive to bacteria. Studies on the nature of the antibacterial antibody will be published in the succeeding reports.

In a study of auto-allergy to skin in disseminated eczema—mostly rapid dissemination from an active eczema on the lower leg to the arms, thighs and face (Whitfield-type)—auto-antibodies cytotoxic for skin were found in only four of eighty-one patients (Parish, Rook & Champion, 1965). A further study was therefore made to test the possibility that such eczema in some patients results from hypersensitivity to bacteria.

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The role of bacteria in eczema has been considered since the late nineteenth century when bacteria were first found in eczematous exudates, although by the turn of the century it was apparent that the eczema vesicles were sterile. More recent attempts to incriminate bacteria as a cause of eczema, mainly by skin testing (Rajka, Korossy & Gozóny, 1953; Storck, 1964; Röckl & Schröpl, 1964), have not been conclusive.

Though bacteria of several genera and species occur on skin, those most frequently colonizing the skin are staphylococci, micrococci and diphtheroids (Marples, 1965, 1969; Somerville, 1969; Noble & Somerville, 1974). The 'unaffected' skin of eczematous persons carries more *Staphylococcus aureus* organisms than does skin of normal persons (Hare & Cooke, 1961; Noble & Davies, 1965; Müller, 1969; Noble, 1971; Wilson, White & Noble, 1971). Numerous bacteria, particularly *Staph. aureus*, colonize moist eczematous lesions (Pillsbury & Nicholas, 1946; Storck, 1948). It is often considered that these organisms are adventitious contaminants colonizing the suitable culture medium without inducing or perpetuating the eczema.

The evidence that allergy to bacteria causes eczema has been based mainly on the results of skin tests, combined with somewhat inconclusive clinical evidence, including response to treatment.

It is generally agreed that patients with eczemas, such as those designated nummular, microbial, coccal, etc. give positive skin tests to staphylococcal antigens more frequently than do control persons (Korossy, 1964; Schröpl, Müller & Röckl, 1964). Baird (1957), in a review of the literature, and Rudzki *et al.* (1964a, b) reported a greater frequency of positive skin tests in 'bacterial'/'microbial' eczema than in 'nummular'/'non-microbial' eczema. The tests have usually been intradermal antigenic injections read at 15 min and 48 h. Delayed sensitivity has also been examined by patch tests read at 48 h. Some positive patch tests with bacterial substances have been attributed to primary irritation (Storck, 1964). Patch tests induce fewer positive reactions than intradermal injections. Almost no tests have been made to study cytotoxic reactions, or reactions induced by antigen–antibody complexes.

Various preparations of staphylococci have been used as skin-test antigens, some of which are shown in Table 1, without any one preparation being particularly active in eczematous patients. Moreover, some of the results reported are likely to be the effects of primary irritation, or an antigen non-specific activation of pharmacological mediators, especially after intradermal injections. The lack of supporting laboratory tests on sera or cells of the patients makes assessment of the results difficult. Furthermore, the association between positive skin tests and 'microbial' eczema is not evidence that the bacterial antigens have contributed in any way to the eczema, and the allergic sensitivity could well result from increased exposure to bacterial antigens absorbed from colonized eczematous skin.

Most living or dead bacteria in the skin are shed with the corneal squames, but some bacterial antigens are absorbed through the epidermis and are taken up by dermal mononuclear cells, as shown by immunofluorescence in this study.

It was thought that this phenomenon could contribute to epidermal cell damage in eczema. Bacteria dying in the stratum corneum release soluble antigens, some of which may diffuse into the stratum Malpighii and become firmly adsorbed to the epidermal cells. The adsorbed bacterial substance is a complete antigen, but the concept of antigen binding corresponds to the hapten or 'Mikrobide' of Röckl (1955a, b), Rajka, Korossy & Gozóny, (1962) and Storck (1964). Should any change in the skin permit passage of antibody and complement through the dermo–epidermal junction, the antibody, reacting with the antigens acquired by the epidermal cells, may induce vacuolation or lysis of the cells.

In this and in three subsequent papers we report the results of a 7-year study of hypersensitivity to bacteria in eczema, and the possibility that it mediates damage and even epidermal cell lysis.

TABLE I. Reports on the results of skin tests with various bacterial antigens on eczematous patients

Antigen	Test	Response % positive: Anaphylactic 15–30 min	Response % positive: Delayed 24–48 h	Reference
Staph. filtrate	I.D.	17·5	26·4	Sipos, 1965
Staph. vaccine	I.D.	20·5	8·6	
Strep. filtrate	I.D.	23·8	13·7	
Strep. vaccine	I.D.	13·2	24·2	
Staph. anatoxin	I.D.	52·9	47·8*	Rudzki *et al.*, 1964 a, b
Staph. nucleoprotein	I.D.	11·5	19·2	
Staph. polysaccharide	I.D.	32·7	23·8	
Staph. broth culture and filtrate Staph. extract in saline	P−		68	Storck, 1948
Staph. albus as above	P−		6	
Strep.-haemolytic as above	P−		24	
Staph. suspension + homogenized skin	P+		60*	Rajka *et al.*, 1953
Staph. + skin suspension Seitz-filtrate	P+		75	
Staph. toxoid	I.D.		41	Korossy, 1964
Staph. toxoid	P−		0	
Staph. filtrate from standard 1·2 g% × 10 conc.	P−		83	Schröpl *et al.*, 1964
Staph. filtrate from standard 1·2 g% × 5 conc.	P−		66	
Staph. protein	P+ & −		38† 54‡	Meyer-Rohn, 1964
Staph. nucleoprotein and protein	P+ & −		27† 38‡	
Staph. polysaccharide and nucleoprotein	P+ & −		51† 75‡	

Test: I.D. intradermal; P− patch without; P+ patch with stripping or scarification.
* Mostly non-eczematous subjects. † 'Non-microbial' eczema. ‡ Microbial eczema.

Patients were examined for hypersensitivity to *Staph. aureus*, *Staph. albus* (micrococci), *Escherichia coli* and *Candida albicans* by intradermal skin tests, and for complement-activating antibodies to some bacterial antigens. Furthermore, in thirty of these patients, samples of eczematous and normal skin were examined by immunofluorescence for immunoglobulins, complement and bacterial antigens. Samples of the skin were also grown in culture, treated with bacterial antigen, and subsequently with the patient's serum containing antibody and with complement to observe any cytotoxic change. The patients' sera were also tested by immunofluorescence and on cultures of normal skin to detect any antibody that was cytotoxic to skin by reacting with skin antigen, as occurs in auto-allergy to skin in Whitfield-type generalized eczema (Parish *et al.*, 1965).

MATERIALS AND METHODS

Tests on eczematous patients

A detailed personal and family history was obtained and full routine examination was made on each of 122 eczematous patients.

Moistened swabs for bacteriology were taken from eczematous and corresponding areas of normal skin, leg ulcers or other external areas of focal infection (e.g. boils, tooth sockets) as well as cultures of urine, nasal washouts and blood where indicated. The results were recorded semi-quantitatively as −, +, + +, + + +. Skin tests were made on all patients. Skin biopsies were taken from normal and eczematous areas of thirty patients for immunofluorescence to detect immunoglobulins and bacterial antigens, and for tissue culture studies. Tests were repeated if further attacks of eczema occurred whether or not there was clinical evidence of infection or whether dissemination recurred. Full blood counts and ESR determinations were discontinued after more than half the cases had uniformly shown no departures from normal values.

Controls. Seventeen patients acted as controls. Ten were volunteers and relatives of the eczematous patients and were, presumably, exposed to a similar bacterial environment as the patient. None had any skin disease, any known septic focus or any history of allergy. Two controls were patients with nodular vasculitis, two with psoriasis and three with acne. Three healthy laboratory workers were additional controls for the skin test antigens.

Intradermal skin tests

Skin tests were carried out by injecting 0·1 ml volumes of antigens, and were all carried out and assessed by one of us (E.W.). Tests were made with Bencard* particulate antigens of *Staph. aureus*, *Staph. albus* and Candida, and with specially prepared (W.E.P.) Lister particulate antigens of *Staph. aureus*, and Micrococci (*Staph. albus*), and Lister soluble antigens of staphylococcal phenol extract (at 10 μg in 0·1 ml) and total extract (0·5 μg in 0·1 ml). These Lister antigens were shown not to elicit reactions in normal persons whose sera contained little specific antibody. An injection of a control diluent was always made.

Whenever possible the intradermal tests were carried out on the volar aspects of the forearms. If this area was too extensively eczematous, the skin of the upper back was tested. If, however, the eczematous eruption was too florid, intradermal testing was postponed.

Skin-test responses were observed on in-patients at 15 min (immediate), 4 h (Arthus-like), 24 h and 48 h, and were recorded as weal, flare and papule. With some out-patients in whom it was not possible to observe all responses, the patient was given a chart to complete. The responses compared with the control were graded −, $\overset{-}{\mp}$, $\mp$, ±, +, + +, + + + for weal, flare and papule, comparable to the assessment of Sipos (1965). The speed of appearance of the initial weal and flare was noted as well as the intensity. Patch tests were also made at the start of the investigations, but were discontinued because so few persons reacted. The same antigens were used as in the intradermal tests, and were applied both with and without scarification.

Preparation of bacterial antigens

The culture of *Staphylococcus aureus* used to prepare the Lister antigens for skin and *in vitro* tests was obtained from the skin of an eczematous patient, HS. It corresponded to the Baird-Parker (1963, 1965) classification type S1, was coagulase positive, fermented glucose aerobically and anaerobically, and formed acid from lactose, maltose and mannitol. It formed Protein A. The two cultures of

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* Bencard Laboratories, Great West Road, Brentford, Middlesex.

'micrococci', F and M1, were obtained from the skin of two other patients and differed in their protein and phenol-extractable antigens. They correspond to Baird-Parker types Sv1 and M1 but are referred to as Micrococci throughout the text in order to avoid confusion possibly resulting in referring to Sv1 as *Staph. albus* or *Staph. epidermidis*.

Particulate antigens were prepared by incubating cultures on nutrient agar in Roux flasks for 2–5 days according to growth. The organisms were washed off in buffered saline, washed once and the centrifuged deposit shaken for 1 h in acetone previously dried over calcium carbonate and cooled to 0°C. This was repeated once, and on the third treatment the acetone was left overnight. The acetone was then removed, and the organisms dried in a vacuum in a desiccator.

The organisms were resuspended in saline, and allowed to equilibrate in suspension, disaggregated by gentle treatment with a homogenizer, and any heavy particles allowed to sediment. Then a concentration of $1\text{–}3 \times 10^7$ particles/ml was prepared in 0·01% merthiolate saline. For skin tests a merthiolate saline solution which had been washed once for 3 min over the surface of a dried nutrient agar plate was prepared as a control solution. The antigens were then dispensed in 1 ml ampoules and sealed. Total extracts were prepared by twice crushing freeze-press (Edebo, 1960) 5 ml volumes of packed washed bacteria grown on nutrient agar, and extracting soluble substances in saline, culture medium or distilled water as required. Solutions in water were freeze dried and dissolved as 1% solutions in the appropriate diluent for the test, and further diluted as required. Some solutions of *Staph. aureus* were toxic for cells *in vitro* and were heated to 56°C for 30 min which abolished the toxicity.

Protein extracts were prepared by treating 10% solutions of the total extract with 10% trichloracetic acid, dissolving the precipitate in the requisite solvent, e.g. culture medium, and dialysing against the medium for 2 days at 4°C.

Phenol-extracts were made by treating packed, living, washed organisms in 45% phenol at 68°C by the method of Westphal (Stacey & Barker, 1960), dialysing the aqueous phase against tap water for 3 days, and treating the extract with 70% ethyl alcohol. The precipitate was dried, and subsequently dissolved in the appropriate solvent. Such extracts contain lipopolysaccharides of Gram-negative organisms, and probably teichoic and some nucleic acids of Gram-positive organisms.

Immunofluorescence tests for antibodies and bacterial antigens

Pieces of normal and eczematous skin were taken from thirty patients. A portion of each sample was snap frozen and sections prepared for immunofluorescence tests. Sections from all samples were examined unfixed. Some sections from fifteen of the thirty patients were fixed in cold dry acetone at 2°C to preserve any uncombined immunoglobulins which would diffuse out during washing. The technique, and preparation of fluorescein or rhodamine-labelled antiglobulins, anti-complement (C3c, C4, C3d) and anti-bacterial antigens have been described previously (Parish, 1970, 1971).

RESULTS

The results of tests on the 122 patients with widespread eczema are considered in this report in the following groups: group G, generalized eczema, sixty-one patients; group N, nummular eczema, forty-six patients; and group O, other forms of eczema, e.g. localized or seborrhoeic, fifteen patients. The classification of the eczemas was according to conventional clinical criteria. The generalized group included cases of Whitfield-type, disseminating from a stasis eczema. The nummular group patients had circumscribed plaques on the backs of the hands or on the arms and legs. The distinction between a typical nummular eczema and a nummular eczema which had disseminated sufficiently to justify its inclusion in the generalized eczema group was often arbitrary. As the investigation was concerned mainly with widespread eczema in which the aetiology was not apparent, cases in which the diagnosis was primary irritant dermatitis, atopic dermatitis, localized hand eczema or lichen simplex, were excluded.

Full blood counts and ESR determinations were found to be normal in all of the first half of the total number of patients, and were therefore discontinued.

Bacterial cultures

The results of all swabs from the skin and other sites of each person were considered together to indicate exposure to bacterial antigens that could be found on the surface of epidermal cells.

Staphylococcus aureus (pyogenes) and micrococci (*Staph. albus*) predominated and were isolated from the skin or nose of most patients. In group G the lesions of thirty-two of the sixty-one persons carried *Staph. aureus*, and seventeen, micrococci; in group N, *Staph. aureus* was isolated from twenty-four and micrococci from twenty-four of the forty-six patients; in group O, *Staph. aureus* was isolated from seven and micrococci from seven of the fifteen patients. The 'normal', unaffected, skin and/or the upper respiratory tract of persons of group G, was colonized by *Staph. aureus* in seven and micrococci in eight; in group N, by *Staph. aureus* in five and micrococci in nine; in group O, by *Staph. aureus* in one and micrococci in three patients.

Other bacteria were found infrequently. Among them were haemolytic streptococci in the lesions of eight, normal skin of one and upper respiratory tract of one person. Non-haemolytic streptococci were found in the lesions of three persons. *Escherichia coli* was cultured from the lesions of three persons and once from normal skin. Pyocyaneus was found in the lesions of three persons and *Bacillus subtilis* in two. Corynebacteria and diphtheroids were not specifically sought.

Skin tests with bacterial antigens

Patients were examined for hypersensitivity to *Staphylococcus aureus*, micrococci (*Staph. albus*), *Escherichia coli* and *Candida albicans* by skin tests. As the subsequent tests on the cytotoxic properties of the anti-bacterial antibodies were mainly restricted to the staphylococcal and micrococcal antigens, the skin-test responses to these two antigens only are reported. The responses (Table 2) are the combined results of tests with the Lister and Bencard antigens; the Bencard *Staph. albus* antigen is included in the designation, Micrococcal.

The results of the skin tests were unrelated to the type and severity of the eczema, or to the antibody class or titre as reported in the third paper of this series.

The most significant findings were the large proportion of persons in the N and O eczema groups who gave immediate responses to both staphylococcal and micrococcal antigens, compared to the proportion of the controls responding to these antigens. A relatively small proportion in all groups responded with 4 h Arthus-like reactions, and the number of persons with delayed sensitivity to both antigens was also small (Table 2).

Persons with 4 h or with delayed-type responses to staphylococcal antigen frequently showed the same response to the micrococcal antigens. The two antigenic preparations were dissimilar in the agglutination tests, so the determinants effecting the *in vivo* responses appeared to differ from those reacting with antibody *in vitro*. The micrococcal antigens did not contain Protein A.

There were differences between the Lister and Bencard antigens. The Lister staphylococcal antigen elicited reactions in sixty-six eczematous persons and the Bencard in forty-three; the Lister micrococcal antigen elicited reactions in forty-nine persons and the Bencard in forty-three.

Immunofluorescence investigations

Unaffected skin from eczematous patients. 'Unaffected' or apparently normal skin from patients with

TABLE 2. Percentage of each type of skin-test response to staphylococcal or micrococcal antigens, in the G, N, and O groups of eczema, and in control persons

			% Responding in each group*			
Skin response	Antigen	Group/ Number/	G 64	N 38	O 11	Controls 20
Immediate	Staph.		21·8	55·2	75	10
	Microc.		26·5	44·7	36·3	20
Arthus-like	Staph.		9·3	0	9·1	5
	Microc.		9·3	0	9·1	0
Delayed	Staph.		3·1	10·5	0	0
	Microc.		3·1	13·1	0	5
Immediate + Arthus-like	Staph.		20·3	7·8	9·1	20
	Microc.		12·5	10·5	18·1	20
None	Staph.		25·0	26·3	6·8	15
	Microc.		34·3	26·3	27·2	15

* The percentage of persons giving a triple immediate–Arthus-like–delayed response, or the double immediate–delayed response are omitted, and accounts for the total percentages being less than 100. Some persons gave similar responses to both antigens.

generalized eczema contained more immunoglobulin, some aggregated or bound, than was found in normal skin of healthy persons as seen in previous studies (Parish, 1971).

Unfixed, washed sections of clinically normal skin from eczematous patients contained immunoglobulins in twelve of the thirty samples, IgG being found in nine and IgM in four (Table 3). In two samples both globulins were found. IgD was found in one of eight samples examined, and complement, as C3c, C3d or C4, in two samples which also contained IgG though not in the same sites. IgG was present in small amounts, showing as stippled fluorescence between epidermal cells, at the dermo–epidermal junction, or in larger, usually intracellular deposits in perivascular lymphatic vessels where it was probably within plasma cells. IgM was in small deposits beneath the dermo–epidermal junction, or as perivascular speckles in the mid dermis. In fixed normal skin, extravascular IgG and IgA were seen diffusely in the upper dermis, as usually found in any normal skin, though no immunoglobulin at all was seen in four samples. IgD was in or on a few small mononuclear cells in the mid dermis.

Eczematous skin. In unfixed, washed sections of eczematous lesions, immunoglobulins were found in eighteen of the thirty samples, in which the control tests showed no evidence of antigen non-specific adherence of the fluorescein-labelled antisera. IgG was present in all eighteen, and was accompanied by IgA in six and IgM in seven patients (Table 3). Five samples contained all three immunoglobulins, and three contained IgD.

IgG tended to be diffuse in the upper dermis and to line the collagen fibres. Irregular deposits were also found in the upper dermis, not particularly associated with blood vessels, and in five samples it was present within occasional epidermal vesicles (Fig. 1), and as those vesicles containing it tended to be infiltrated by a few neutrophils and mononuclear cells, it is thought that the IgG had diffused into the lesion as a late inflammatory change. IgG was also found on the skin surface even in the absence of vesicles. IgM was found in sites similar to those containing IgG, but it was more frequently present on the skin surface. It was found within a few vesicles in two patients. IgA tended to be diffuse in the

TABLE 3. Incidence of immunoglobulins, complement, and bacterial antigens in sections of 'normal' and eczematous skin from thirty eczematous patients

Skin condition	Sections treatment	Samples total no.	No. samples nil findings	No. of samples containing Globulins					Bacterial antigens				
				IgG	IgA	IgM	IgD	C	*Staph.*	*Mic.*	*Strep. p.*	*Strep. v.*	*E. coli*
Normal	Unfixed	30	18	9	3	4	1/8	2	1	3	0	0	0
Normal	Fixed	15	4	11	5	4	2/8	0	0	0	0	0	0
Eczematous	Unfixed	30	12	18	6	7	3/8	3	7	9	1	4	2
Eczematous	Fixed	15	1	14	4	6	6/8	5	0	3	1	0	0

C = complement (C3c, C3d, C4).

Bacterial antigens: *Staph. aureus*, *Micrococcus*, *Strep. pyogenes* group A, *Strep. viridans*, *Esch. coli*.

IgD in unfixed sections are number containing IgD/number examined.

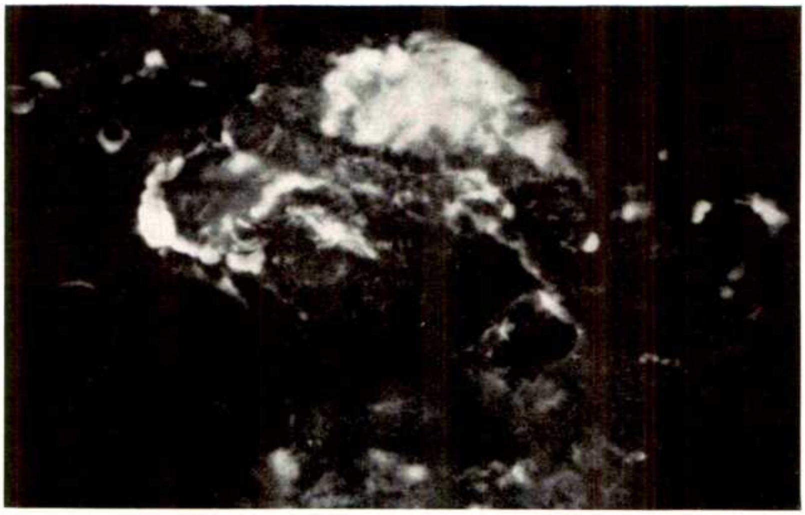

FIGURE 1. Unfixed washed section of eczematous lesion showing immunofluorescent detection of IgG in diffuse deposits in the upper dermis, and within an epidermal vesicle. Small amounts are also present on the skin surface. The test was specific, and the reaction was not inhibited by albumin, by IgG Fc, or by anti-IgM, but was blocked by unlabelled anti-IgG.

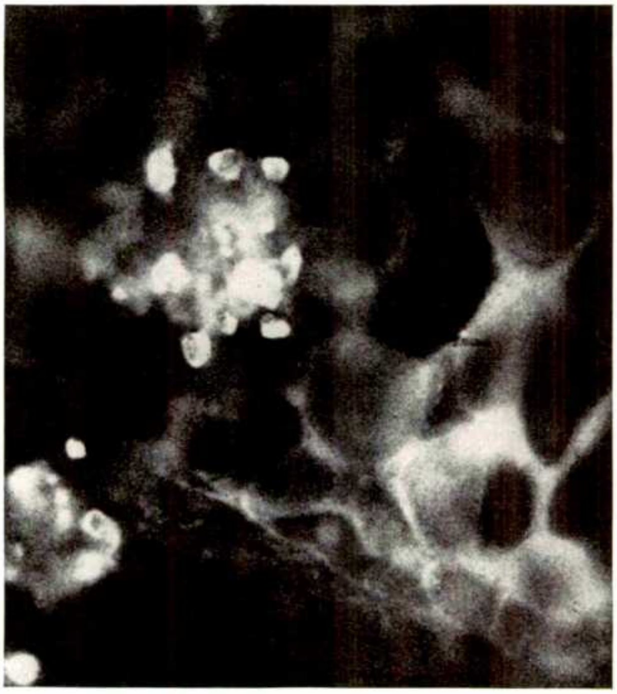

FIGURE 2. Unfixed washed section of edge of eczematous lesion. Groups of mononuclear cells (? large lymphocytes) stained by immunofluorescence for IgD. A reaction not inhibited by albumin, IgG Fc, anti-IgG or anti-IgM.

upper dermis, and in slightly greater concentrations round some venules and irregularly beneath the dermo–epidermal junction.

IgD, in three of eight unfixed sections, was mainly intracellular in occasional perivascular cells of the upper dermis, and in two patients was also in the epidermis in groups of mononuclear cells, which on subsequent staining appeared to be large lymphocytes (Fig. 2).

Fixed eczematous skin had diffuse IgG in the dermis of fourteen of fifteen samples, and in all fourteen there were linear traces between epidermal cells, and speckles within epidermal cells particularly near vesicles. In five samples speckles were also found within nuclei of occasional epidermal cells, so consistently on the inner surface of the nuclear membrane or on chromatin strands as to indicate physiological deposition rather than an artifact. IgG and IgM were found on the surface of the stratum corneum. IgM also occurred diffusely in the dermis, and as linear traces between epidermal cells. IgA was diffusely and irregularly present in the upper dermis, particularly beneath the dermo–epidermal junction. IgD was intra- or pericellular, or faint extra-cellular traces were seen round any groups of IgD-containing cells.

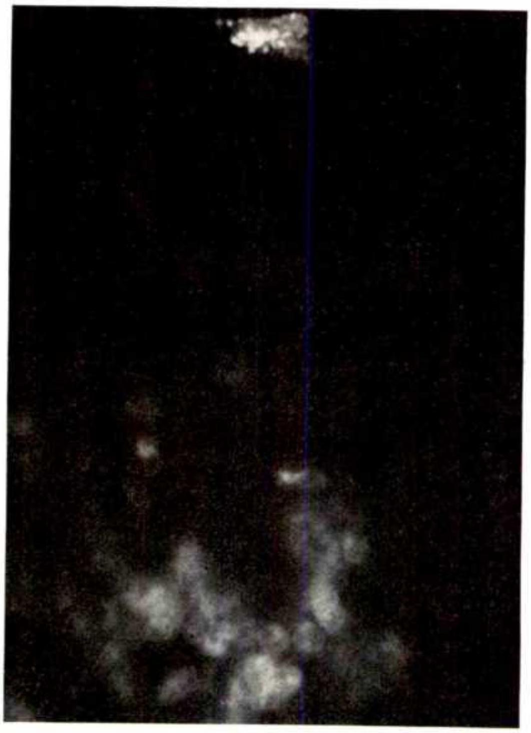

FIGURE 3. Unfixed section of normal skin from eczematous person. Micrococci (*Staph. albus*) organisms on the skin surface, and the same antigen diffusely within the cytoplasm of mononuclear cells in the upper dermis. Evidence of percutaneous absorption.

Bacterial antigens. Staphylococcal and micrococcal antigens were occasionally found on the skin surface or in the epidermis of normal skin, and more frequently in eczematous skin (Table 3). *Streptococcus viridans* antigen and *Escherichia coli* antigen were also found. In two samples of normal skin and three of eczematous lesions, foci of micrococcal (*Staph. albus*) organisms on the surface had released antigen which had apparently penetrated the epidermis and been taken up diffusely within the

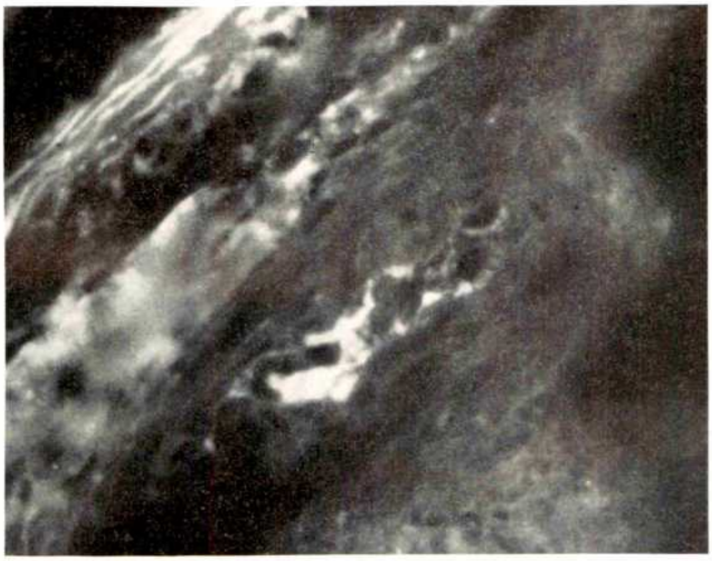

FIGURE 4. Unfixed section from eczematous skin. Micrococcal (*Staph. albus*) antigen between stratum corneum and stratum Malpighii cells, and fine particles within epidermal cells (mid-right).

cytoplasm of groups of mononuclear cells in the dermis (Fig. 3). The antigen within the dermal cells corresponded exactly with that on the skin surface, reacting with the same indicator antisera, and the reaction being inhibited (blocked) by the same antisera. The antigen in the dermis did not represent a focus of infection because it was very diffuse and easily distinguishable from the particulate antigen of cocci on the skin surface (Fig. 3), and there was no infiltration by neutrophils such as would occur during infection. Staphylococcal (aureus) antigen was also found on the surface of some eczematous skin, and homogeneous antigen appeared to diffuse down into the stratum Malpighii. The diffusion is unlikely to be an artifact, because small deposits were found intracellularly in the mid to upper dermis. In some lesions, large deposits of micrococcal antigen were found round and occasionally within epidermal cells (Fig. 4).

The immunofluorescence technique was subsequently shown to be an insensitive method of detecting bacterial antigen in epidermis. Some skin samples that were heavily contaminated with staphylococci, micrococci or *Streptococcus viridans*, showed little or no bacterial antigen by immunofluoresence, though the surface squames, and even cryostat cut sections, yielded numerous bacterial colonies. When cultured, these bacteria combined with the appropriate fluorescein-labelled antisera, so the failure to detect more organisms in the skin sections by immunofluorescence may have been due to the bacteria being shielded or masked in the skin section, or to individual organisms being too difficult to distinguish in a section, but forming a discrete colony on culture media.

DISCUSSION

This study confirms the now well known presence of *Staph. aureus* and other organisms on the skin lesions of patients with disseminated and other eczemas. It also confirms the high incidence of positive skin tests to the bacterial antigens. These alone are no evidence that allergic sensitivity to bacteria plays any part in initiating or even aggravating the eczema.

Some features of the findings of the skin tests are of particular interest. The responses reflect some susceptibility in the patients, not necessarily allergic. The Lister and Bencard antigens contained similar numbers of particles, and approximately equivalent amounts of antigen determined by antibody neutralization tests. Both antigens were non-irritant when examined by titration on three normal persons, and only a small proportion of twenty control normal persons showed responses.

The skin-test responses most frequently observed were immediate, particularly in the G and N groups, and immediate followed by Arthus-like. There were surprisingly few delayed hypersensitivity responses, though this type of response is most frequently reported in the literature (Table 1). It is still to be determined whether the delayed responses reported so frequently are true allergic reactions, or are manifestations of slowly developing Shwartzman-like reactions, or antigen non-specific activation of lymphocytes. Substances in staphylococcal culture media are antigen non-specific mitogens which activate lymphocytes *in vitro*, though their activity *in vivo* is still to be determined.

The nature of the immediate skin-test responses is still to be investigated. There is no evidence yet that they are IgE-mediated anaphylactic responses, but no *in vitro* tests for this class of antibody were made. However, no IgE anti-bacterial antibodies were found by monkey cutaneous passive anaphylaxis, though it is possible to elicit immediate reactions in man without sufficient IgE antibody in the serum to confer anaphylactic sensitivity on monkey skin. The responses are unlikely to result from a reaction with IgG or IgM antibody and complement-mediating histamine release from mast cells, leukocytes or platelets, because there was no relation between the presence and amount of IgG or IgM specific antibody and the response. It is, however, conceivable that some people are hypersusceptible to histamine release mediated by small amounts of antibody.

There is a similar problem in assessing the Arthus-type responses which occur 2–4 h after testing, and appear as indurated lesions. The details of these will be published in the second paper of this series, but, as in the immediate responses, there was no relation between the occurrence and severity of the reaction with the presence and amount of specific IgG or IgM antibody, or the ability of the antibodies to fix complement, which is an essential feature of the classical Arthus response.

In two subsequent reports of this study evidence is adduced that the immediate and Arthus-like responses are not induced by Protein A. The staphylococcal phenol extract antigens and the micrococcal test antigens did not contain this substance, though eliciting both types of response.

The immunofluorescence tests showed that lesional and 'unaffected' skin of eczematous patients contains immunoglobulins, particularly IgG. Some of the globulin is aggregated and not easily washed from the section, but this is believed to be due to inflammatory adherence and is not evidence of an allergic reaction. Vesicles very occasionally contained IgG or IgM, and this too is considered to represent inflammatory diffusion of immunoglobulins. The finding of IgG and IgM on the skin surface, and of IgG within and round epidermal cells in fixed sections, indicates that this immunoglobulin diffuses through the epidermis, and presumably could form complexes with appropriate bacterial antigens. It is noteworthy that 'unaffected' clinically normal skin of patients with disseminated eczema contained more globulins than was found in normal healthy persons, indicating that even 'normal' skin is physiologically changed in these persons.

The numerous micro-organisms in or on the skin are mainly shed with the squames, as doubtless are most of the substances from dead bacteria. However, our immunofluorescence tests showed that some bacterial antigens, particularly *Staph. aureus* and micrococci (*Staph. albus*), on the skin surface are absorbed into the epidermis and dermis, and possibly induce local formation of antibody. Nevertheless, though bacterial antigen, immunoglobulin and complement were found between epidermal cells, they rarely occurred at the same sites, and there was no evidence of a cytotoxic reaction. In some sections, bacterial antigen and immunoglobulin are found between apparently normal epidermal cells.

When the results of bacterial culture and immunofluorescence tests on each piece of skin are compared, it is evident that culture is the better method of detecting bacteria in skin, because skin shown by culture to be contaminated often had no detectable organisms when examined by immunofluorescence. Culture detects organisms sampled from an area of skin, or a relatively large volume of excised skin, compared to the five or seven thin sections examined by immunofluorescence. However, immunofluorescence has an advantage in that it can detect antigens released from dead bacteria. In this study bacterial antigen was shown to be absorbed through the skin of persons with disseminated eczema, and to be bound to some epidermal cells, potentially predisposing them to the cytotoxic activity of specific antibody and complement, or antigen-actuated lymphocytes.

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REFERENCES

Baird, K.A. (1957) Allergy to bacterial products. A review of the literature. *Annals of Allergy*, **15**, 174.

Baird-Parker, A.C. (1963) A classification of micrococci and staphylococci based on physiological and biochemical tests. *Journal of General Microbiology*, **30**, 409.

Baird-Parker, A.C. (1965) Staphylococci and their classification. *Annals of the New York Academy of Science*, **128**, 4.

Edebo, L. (1960) A new press for the disruption of micro-organisms and other cells. *Journal of Biochemical and Microbiological Technology and Engineering*, **2**, 453.

Hare, R. & Cooke, E.M. (1961) Self-contamination of patients with staphylococcal infections. *British Medical Journal*, **ii**, 333.

Korossy, S. (1964) Einfluss einer innerlichen Anti-oder Anatoxin-applikation auf die ekzematöse Reagibilitat gegen Mikroben und Mikrobine. *Archiv für klinische und experimentelle Dermatologie*, **219**, 845.

Marples, M.J. (1965) *The ecology of human skin.* Charles C. Thomas, Springfield, Illinois.

Marples, M.J. (1969) The normal flora of the human skin. *British Journal of Dermatology*, **81**, Suppl. 1, 2.

Meyer-Rohn, J. (1964) Über die Natur der mikrobiellen ekzematogenen Stoffe. *Archiv für klinische und experimentelle Dermatologie*, **219**, 842.

Müller, E. (1969) Zur Ökologie von Staphylococcus aureus-auf der menschlichen Hautoberfläche. V. Staphylococcus aureus nach künstlicher Verimpfung auf die Hautoberfläche nach Äther-Extraction, von hautkranken Versuchspersonen und während innerlicher Antibioticagabe. *Archiv für klinische und experimentelle Dermatologie*, **233**, 376.

Noble, W.C. (1971) The contribution of individual patients to the spread of infection. *British Journal of Dermatology*, **85**, 24.

Noble, W.C. & Davies, R.R. (1965) Studies on the dispersal of staphylococci. *Journal of Clinical Pathology*, **18**, 16.

Noble, W.C. & Somerville, D.A. (1974) *Microbiology of human skin.* W.B. Saunders, London.

Parish, W.E. (1970) Complexes of bacterial antigens with IgG or IgM antibodies in cutaneous vasculitis. In: *International Symposium on Immune-complex Diseases, Spoleto, Italy*, p. 98. Carlo Erba, Milan.

Parish, W.E. (1971) Studies on vasculitis. I. Immunoglobulins, β_1C, C-reactive protein and bacterial antigens in cutaneous vasculitis lesions. *Clinical Allergy*, **1**, 97.

PARISH, W.E., ROOK, A.J. & CHAMPION, R.H. (1965) A study of auto-allergy in generalized eczema. *British Journal of Dermatology*, **77**, 479.

PILLSBURY, D.M. & NICHOLS, A.C. (1946) Bacterial flora of the normal and infected skin: an evaluation of various methods of performing skin cultures. *Journal of Investigative Dermatology*, **7**, 365.

RAJKA, E., KOROSSY, S. & GOZÓNY, M. (1953) Künstliche kokkogene Sensibilisierung. *Dermatologische Wochenschrift*, **128**, 1117.

RAJKA, E., KOROSSY, S. & GOZÓNY, M. (1962) *Das mikrobielle Ekzem*. Ungarissche Akademie Wissenschrift, Budapest.

RÖCKL, H. (1955a) Untersuchungen zur Klinik und Pathogenese des mikrobiellen Ekzems. *Hautarzt*, **6**, 532.

RÖCKL, H. (1955b) Untersuchungen zur Klinik und Pathogenese des mikrobiellen Ekzems. VI. Pathogenese des mikrobiellen Ekzems und der Mikrobide. *Hautarzt*, **7**, 304.

RÖCKL, H. & SCHRÖPL, F. (1964) Die Bedeutung von Mikroben für die Chronizität des Ekzems. *Archiv für klinische und experimentelle Dermatologie*, **219**, 862.

RUDZKI, E., FILIPOWICZ-BANACHOWA, A., MACIEJOWSKA, E., MOSKALEWSKA, K. & BLASZCZYK, M. (1964a) Spätallergie auf Streptokokken und Staphylokokken bei Menschen und experimentell infizierten Kaninchen. *Archiv für klinische und experimentelle Dermatologie*, **220**, 547.

RUDZKI, E., MOSKALEWSKA, K., MACIEJOWSKA, E. & BLASZCZYK, M. (1964b) Frühallergie auf streptokokken und staphylokokken bei Menschen und experimentell infizierten Kaninchen. *Archiv für klinische und experimentelle Dermatologie*, **220**, 535.

SCHRÖPL, F., MÜLLER, E. & RÖCKL, H. (1964) Experimentelle untersuchungen zur Pathogenese des mikrobiellen Ekzems. IV. Epicutantests mit Cellophankulturfiltrat von Staphylococcus aureus (haem.) am Menschen auf normaler Haut. *Archiv für klinische und experimentelle Dermatologie*, **218**, 312.

SIPOS, K. (1965) Analyse der intracutanen Reaktionen von Pyokokkenantigen. *Archiv für klinische und experimentelle Dermatologie*, **222**, 430.

SOMERVILLE, D.A. (1969) The effect of age on the normal bacterial flora of the skin. *British Journal of Dermatology* **81**, Suppl. 1, 14.

STACY, M. & BARKER, S.A. (1960) *Polysaccharides of Micro-organisms*. Clarendon Press, Oxford.

STORCK, H. (1948) Experimentelle Untersuchungen zur Frage der Bedeutung von Mikroben in der Ekzemgenese *Dermatologica (Basel)*, **96**, 177.

STORCK, H. (1964) Über experimentelle Erzeugung von Ekzemen mit Mikroben und Mikrobinen. *Archiv für klinische und experimentelle Dermatologie*, **219**, 834.

WILSON, P.E., WHITE, P.M. & NOBLE, W.C. (1971) Infections in a hospital for patients with diseases of the skin. *Journal of Hygiene (Cambridge)*, **69**, 125.

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A familial study of acne and eczema

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SUMMARY

The incidence of eczema was assessed in acne patients and their first degree relatives and compared with control (non-acne) subjects and their relatives. It was found that there was a statistically decreased incidence of endogenous eczema in the acne patients.

It has been my subjective clinical impression that eczema occurs less frequently in acne patients than in subjects without acne, and that acne patients seldom give a family history of eczema. The following study was undertaken to examine this hypothesis.

METHOD

An effective study could only be achieved by assessing the incidence of different types of eczema in acne patients and their first degree relatives, and by comparing the results with the incidence of eczema in control, acne-free subjects and their families. There was a ready source of clinical material available among the acne patients who had been referred for treatment to the Dermatology Outpatient Department, and only they were accepted as acne subjects in this study. All those included in the study had moderate to severe acne with comedones, numerous papules and often pustules; the prerequisite was unquestionable clinical acne. The age-matched control subjects, i.e. non-acne sufferers, were selected from schools and colleges in the Southampton area and were aged from 14 to 20 years. All students seen were questioned and examined for acne. In choosing the control subjects it was found that virtually no one was completely free from comedones, and they were deemed to be controls if they did not exceed a few comedones on the nose, chin and forehead, and up to two papules. Anyone with more lesions was not accepted as a control subject and was eliminated from further study. A clear clinical distinction between controls and acne subjects was ensured.

All the acne and control patients were white and intrinsically British (as judged by skin colour, facial appearance and family history) to exclude differences in ethnic groups.

Controls, acne patients and the first degree relatives of both groups were subjected to the same interview and extensive examination. The incidence and type of eczema were assessed in all groups. For the purpose of this study eczema was divided into several categories: endogenous eczema, exogenous dermatitis (primary irritant and allergic contact), seborrhoeic dermatitis, varicose eczema and peri-oral dermatitis. Endogenous eczema was classified into atopic dermatitis, pompholyx and nummular eczema. Only endogenous eczema showed a significant difference in incidence in acne and control groups. The presence or absence of eczema in both groups was established by interview and physical examination of the subjects and by corroborative questioning of the parents. Where possible all patients' dermatological histories were checked with hospital and General Practitioner records.

RESULTS

The incidence of endogenous eczema in a total number of 210 acne patients was 3·8% compared with an incidence of 19·0% in the 116 controls. This is a highly significant difference ($P < 0{\cdot}001$).

Initially 210 acne patients were seen but some had to be eliminated from the study because the family was not available for assessment due to inevitable family separations. This reduced the number of acne patients to 127 but the incidence of endogenous eczema in this group, when compared with the age-matched control subjects, still showed the same highly significant difference (Table 1).

TABLE 1. The incidence of endogenous eczema in total subjects (both sexes)

	Endogenous eczema	Total	%
Acne subjects	4	127	3·2
Non-acne subjects (controls)	22	116	19·0

$P < 0{\cdot}001$.

The incidence of endogenous eczema in fifty-five male acne subjects was 1·8% compared with an incidence of 20·8% in fifty-three control male subjects ($P < 0{\cdot}005$). In females the incidence was 4·2% in seventy-two acne subjects but 17·5% in sixty-three control females ($P < 0{\cdot}025$).

The first degree relatives (parents and siblings) of the acne patients and the control subjects were then studied. This entailed visiting the families in their own homes; to get parents and siblings of subjects together at one time entailed considerable organization. Where siblings lived outside the area, an appointment was made for holiday times or on the occasion of special family gatherings. The families were seen using the single blind technique, i.e. the investigator did not know whether they were the families of acne patients or control subjects. Clearly it was not always possible to take this to its ultimate limit as the relatives themselves knew which group they fell into and sometimes intimated this. There was a significantly higher incidence of endogenous eczema in the parents of control subjects as compared to the parents of acne subjects, with an incidence of endogenous eczema of 10·5% in the parents of control subjects compared with 2·5% in the parents of acne subjects (Table 2).

This clear-cut difference is still evident when the results are broken down into the parents of male and female subjects. The incidence of endogenous eczema in the 109 parents of male acne subjects

TABLE 2. The incidence of endogenous eczema in the parents of total subjects (both sexes)

	Endogenous eczema	Total	%
Parents of acne subjects	6	243	2·5%
Parents of non-acne subjects (controls)	23	220	10·5

$P < 0{\cdot}001$.

was 2·8% compared with 11·9% in 101 parents of male controls ($P < 0{\cdot}025$). The incidence in 134 parents of female acne subjects was 2·2% but 9·2% in 119 parents of female controls ($P < 0{\cdot}05$).

There was also a substantial difference in the incidence of endogenous eczema in the siblings of acne subjects compared to the siblings of controls. Of 236 siblings of acne subjects there were only ten cases of endogenous eczema but out of 202 siblings of control subjects there were twenty-seven cases (Table 3).

When they were divided into the siblings of male and female subjects the significant difference was still evident for the siblings of male subjects. 5·1% of ninety-nine siblings of acne male subjects had endogenous eczema compared with an incidence of 16% in 100 siblings of male controls ($P < 0{\cdot}025$). Although the incidence of endogenous eczema in the siblings of female controls was virtually three times that of siblings of acne females, its significance was marginal. Five out of 137 siblings of acne females, compared with eleven out of 102 siblings of female controls had endogenous eczema. ($\chi^2 = 3{\cdot}6910$. $0{\cdot}05 < P < 0{\cdot}1$).

TABLE 3. The incidence of endogenous eczema in the siblings of total subjects (both sexes)

	Endogenous eczema	Total	%
Siblings of acne subjects	10	236	4·2
Siblings of non-acne subjects (controls)	27	202	13·4

$P < 0{\cdot}005$.

Thus, all the results indicate that there is a reduced incidence of endogenous eczema in acne patients and their families compared with control subjects and their families. To determine whether the converse is true a separate study was undertaken. Patients in the acne-prone age group who attended Dermatology Outpatients with endogenous eczema, were assessed for acne. It was found that only 8·6% had clinical acne vulgaris whereas over 50% of students at the schools and colleges had clinical acne (i.e. comedones and over five papules).

There is an accepted association between atopic dermatitis and asthma, and it is speculatively significant that the incidence of asthma in acne patients was found to be much lower than in the control subjects. Of 127 acne subjects there were three cases of asthma compared with fourteen cases of asthma out of 116 control subjects ($P < 0{\cdot}01$).

DISCUSSION

Several authors have suggested allergy as a factor in the aetiology of acne: to food (Cunningham & Mendenhall, 1936); chocolate (Baer & Witten, 1960); endogenous hormones (Urbach & Gottlieb, 1946; Yasuda, 1963); and bacteria (Lorincz, 1963). Stokes & Sternberg (1939) felt that the allergic state in its various manifestations was important in the aetiology of acne. They reported the familial and hereditary background of the allergic state present in 33% of 175 cases of acne 'in which a history adequate for study was available'. Unfortunately they did not give their criteria for diagnosing the allergic state or have a control group for comparison. Also they relied on a family history and did not assess the relatives themselves. It is also more likely that a positive history was available for study than

TABLE 4. Incidence of different types of endogenous eczema

	Acne females	Control females	Parents of acne females	Parents of control females	Siblings of acne females	Siblings of control females	Acne males	Control males	Parents of acne males	Parents of control males	Siblings of acne males	Siblings of control males
Atopic eczema	2	9	1	3	3	7	1	10	1	4	3	12
Nummular eczema	1	2	2	5	2	3	0	1	1	8	2	3
Pompholyx	—	—	0	3	0	1	0	0	1	0	0	1
Total	3	11	3	11	5	11	1	11	3	12	5	16

a negative—thus giving a falsely elevated percentage. All these factors probably contributed towards their high figures.

Way (1960) found in almost 1000 cases of acne that about 8% 'suffered from various forms of allergy'. Again, however, the criteria for diagnosing allergy were not specified.

In this study the incidence of endogenous eczema was significantly lower in the acne subjects and their first degree relatives compared with the control subjects and relatives. In view of the fact that acne vulgaris is closely related to sebum excretion it is tempting to attribute these clinical findings partially to a difference in sebum excretion in patients with endogenous eczema. This postulate is supported by work done by Rajka (1975) who found that sebum excretion is significantly lower in patients with atopic dermatitis as compared with age-matched controls. In both sexes he found that the sebum level in controls was virtually double that found in atopic dermatitis patients.

Rajka (1975), Mustakallio *et al.* (1967) and Wheatley (1965) have found that lipids from sebaceous glands (wax esters, triglycerides and squalene) were decreased in patients with atopic dermatitis. Wax esters and squalene in particular have been incriminated by other authors (Cotterill *et al.*, 1972) in the aetiology of acne, by causing blockage of the pilo-sebaceous ducts. In infants with atopic dermatitis there are not only fewer sebaceous glands, but also fewer secretory cells in these glands.

These factors may contribute to the relatively low incidence of endogenous eczema in acne patients.

CONCLUSION

The results of this study indicate that there is an inverse relationship between acne vulgaris and endogenous eczema.

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REFERENCES

Baer, R.L. & Witten, V.H. (1960) *1959–1960 Year Book of Dermatology*, pp. 7–32. Year Book Publishers, Chicago.

Cotterill, J.A., Cunliffe, W.J., Williamson, B. & Bulusu, L. (1972) Further observations on the pathogenesis of acne. *British Medical Journal*, iii, 444.

Cunningham, T.D. & Mendenhall, J.C. (1936) Observations on acne and allergy. *Journal of Allergy*, 7, 378.

Lorincz, A.L. (1963) Pathogenic factors in acne vulgaris. *Illinois Medical Journal*, 124, 418.

Mustakallio, K.K., Kustala, U., Piha, J. & Nieminen, E. (1967) Epidermal lipids in Besnier's prurigo (atopic eczema). *Annales Medicinae Experimentalis et Biologiae Fenniae*, 45, 323.

Rajka, G. (1975) *Atopic Dermatitis*, pp. 109–111. Saunders, London.

Stokes, J.H. & Sternberg, T.H. (1939) A factor analysis of the acne complex with therapeutic comment. *Archives of Dermatology and Syphilology*, 40, 345.

Urbach, E. & Gottlieb, P.M. (1946) *Allergy*, p. 786. Grune & Stratton, New York.

Way, S.C. (1960) X-rays vs other modalities in the treatment of acne vulgaris. *Archives of Dermatology*, 81, 103.

Wheatley, V.R. (1965) Secretions of the skin in eczema. *Journal of Paediatrics*, 66, (Part 2), 200.

Yasuda, T. (1963) The skin and hormones. *Journal of Therapy (Tokyo)*, 45, 1679.

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Regression of a hamster melanoma with intralesional *Corynebacterium granulosum*

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SUMMARY

The present study was undertaken to compare the antitumour effects of intralesionally administered *Corynebacterium granulosum*, *Corynebacterium parvum*, BCG and saline on a hamster melanoma. Forty-eight golden hamsters were inoculated subcutaneously with Fortner's melanotic melanoma no. 1. Twenty-four animals bore palpable tumours which were left *in situ*. The remaining twenty-four animals bore palpable tumours which were excised. No non-excised group animal showed primary tumour regression. In the excised group, the tumours generally recurred at the excision site in the *C. parvum*, BCG and saline-treated animals. By contrast, the incidence of recurrence was significantly reduced by intralesional injections of *C. granulosum*.

In previous work, golden hamsters bearing Fortner's melanotic melanoma no. 1 received *Corynebacterium granulosum* (*C. granulosum*) by intra-tumour injection after the inoculation and appearance of small (≤5 mm in diameter) palpable tumours. The primary tumours and their pulmonary metastases regressed (Paslin, Dimitrov & Heaton, 1974). Unpublished work with this hamster–melanoma model suggested that the occurrence of regression was dependent on tumour size at the time treatment with *C. granulosum* was begun.

The present study was designed to compare the effect of *C. granulosum* in hamsters whose large (>10 mm in diameter) primary tumours were excised with those whose large primary tumours were left *in situ*. In addition, the efficacy of *C. granulosum* was compared with that of *C. parvum*, *Bacillus Calmette Guerin* (BCG) and saline in animals with excised and unexcised tumours.

MATERIALS AND METHODS

Fortner's melanotic melanoma no. 1 has been maintained by serial subcutaneous (s.c.) trocar transplantations in a colony of golden hamsters. This tumour grows as a subcutaneous (s.c.) mass and regularly metastasizes to the lungs (Paslin, 1973b).

The immune stimulants used were: *C. granulosum* containing 2 mg/ml heat-killed formalin-fixed organisms in normal saline (*C. granulosum* was supplied by Professor Lucien Israel and was prepared by the Pasteur Institute, Paris, France); *C. parvum* containing 7 mg/ml formalin-killed organisms in normal saline (Wellcome Reagents Division, Research Triangle Park, North Carolina); BCG containing 10^8 organisms lyophilized and reconstituted in distilled water (Chicago Research Labs., University of Illinois, Chicago, Illinois).

Melanoma tissue was harvested from the peripheral tumour mass of three donor animals. The tissue was minced in cold Hanks' solution and was agitated in the presence of trypsin 0·25% for ½ h. The cells were pressed through cheesecloth, washed with fresh Hanks' solution, centrifuged at 1900 rev/min and suspended in Eagles MEM. More than 95% of melanoma cells excluded trypan blue 0·1%. The cells were concentrated to $3{\cdot}5 \times 10^6$ cells/0·1 ml.

Each of forty-eight female 2-month-old hamsters received 0·1 ml of tumour cell suspension s.c. in the right flank. When the tumours had grown to $\geqslant 10$ mm in longest dimension by caliper measure, the hamsters were divided into two groups: a non-excised group of twenty-four animals and an excised group also of twenty-four animals. In the non-excised group, injections of each stimulant were begun into the base of the tumour mass. In the excised group, tumour removal was complete by macroscopic examination. The tumour base and the underside of adjacent skin flaps were carefully inspected for residual tumour. The term 'excisional' is here defined as macroscopically complete local removal of the primary tumour. Wounds were closed with sutures to approximate the edges. Injections of each stimulant into the site of excision were begun 2 days after excision of the primary tumour mass. In both groups, injections were given weekly for 5 weeks. Table 1 lists the groups and the dosages used.

The incidence of tumour regression in the non-excised group, the incidence of tumour regrowth in the excised group, and the number of pulmonary metastases were measured. Only macroscopic metastases clearly identifiable as such were counted.

TABLE 1. Experimental design

Material	Dose (ml weekly × 5 weeks)	No. of animals
Non-excised group		
Saline	1/2 IT	6
C. granulosum 2 mg/ml	1/2 IT	6
C. parvum 7 mg/ml	1/2 IT	6
BCG 10^8 orgs/ml	1/6 IT	6
Excised group		
Saline	1/2 ES	6
C. granulosum 2 mg/ml	1/2 ES	6
C. parvum 7 mg/ml	1/2 ES	6
BCG 10^8 orgs/ml	1/6 ES	6

IT = intratumour; ES = excisional site.

RESULTS

The incidence of regression of the primary s.c. melanomas is given in Table 2. In the non-excisional group no animal had regression of its tumour. In the excisional group, one BCG-treated animal died too early to determine the presence or absence of tumour regrowth. Each of the remaining five animals treated with BCG had regrowth of its tumour. Among the six animals receiving *C. parvum*, five had regrowth of their tumours. Among the six animals receiving saline, five developed regrowth of

TABLE 2. Incidence of primary melanomas

Material	Primary melanomas	
	No. with	No. without
Non-excised		
Saline	6	0
C. granulosum	6	0
C. parvum	6	0
BCG	6	0
Excised		
Saline	5	1
C. granulosum	2	4
C. parvum	5	1
BCG	5	0

Fisher's exact method comparison of excised saline and excised *C. granulosum*, $P = 0{\cdot}121$. Fisher's exact method comparison of non-excised *C. granulosum* and excised *C. granulosum*, $P = 0{\cdot}030$.

the primary tumours. In the one saline-treated animal whose primary tumour failed to regrow, it is likely that the primary tumour was completely excised without microscopic foci of tumour cells left in the wound (see Discussion). Among the six animals receiving *C. granulosum*, two had regrowth of melanomas and four remained free of tumour. A Fisher's exact method comparison of the excised saline with the excised *C. granulosum* subgroups gives a P value of 0·121. Comparison of the excised *C. granulosum* with the non-excised *C. granulosum* subgroups gives a P value of 0·030.

The incidence of animals free of macroscopic pulmonary metastases in non-excisional and in excisional groups is given in Table 3. In this series of experiments, the s.c. melanomas did not in-

TABLE 3. Incidence of macroscopic pulmonary metastases

Material	Macroscopic pulmonary metastases		
	No. lungs studied	No. with	No. without
Non-excised			
Saline	6	5	1
C. granulosum	6	5	1
C. parvum	5	3	2
BCG	6	5	1
Excised			
Saline	5	3	2
C. granulosum	6	2	4
C. parvum	4	3	1
BCG	4	4	0

There was no significant difference among the subgroups.

variably develop macroscopic pulmonary metastases by the time of autopsy (15 weeks after melanoma inoculation)—a finding which differs from some prior work with this tumour system (Paslin, 1973a). In the non-excisional animals, twenty-three of twenty-four animals at autopsy had lungs which were suitable for study. Unsuitable lungs were lungs too necrotic (autolysed) to allow accurate inspection. Of the twenty-three animals five had lungs that lacked macroscopic metastases. There was no significant difference in the incidence of pulmonary metastases among the non-excisional subgroups: $P = 0{\cdot}421$ (Fisher's exact method).

In the excisional animals, nineteen of twenty-four animals at autopsy had lungs that were suitable for study. Of the nineteen animals, seven had lungs which lacked macroscopic pulmonary metastases. Four of these seven animals had been treated with *C. granulosum*. Two of the seven had received saline and one of the seven had been treated with *C. parvum*. These differences are not statistically significant, $P = 0{\cdot}07$ (Fisher's exact method). Yet it is important to point out that the two saline-treated animals without macroscopic pulmonary metastases nonetheless had regrowth of their primary tumours, whereas the four *C. granulosum*-treated animals and the one *C. parvum*-treated animal without pulmonary metastases did not have regrowth of their primary tumours. Serial sections were made of the lungs of the two saline-treated animals. Numerous microscopic metastases were found.

TABLE 4. Incidence of primary melanomas and macroscopic pulmonary metastases

Materials	Primaries and metastases	
	No. with	No. without
Non-excised		
Saline	6	0
C. granulosum	6	0
C. parvum	6	0
BCG	6	0
Excised		
Saline	6	0
C. granulosum	2	4
C. parvum	5	1
BCG	6	0

Fisher's exact method comparison of excised saline and excised *C. granulosum*, $P = 0{\cdot}030$.

A tabulation of animals free of both primary tumours and of macroscopic pulmonary metastases is given in Table 4. A significant difference in the incidence of animals entirely free of macroscopic tumour is found between the excised *C. granulosum* and the excised saline subgroups, $P = 0{\cdot}030$ (Fisher's exact method).

DISCUSSION

The results indicate that tumour excision is a critical determinant of the response of the host animals to *C. granulosum*. Animals whose large tumours were not excised had no regression of their tumours irrespective of immune stimulant used. Animals whose large tumours were excised had small numbers of tumour cells left in the wound, as evidenced by the regrowth of tumour which occurred in fifteen of seventeen *C. parvum*, BCG, and saline-treated animals.

Animals whose melanomas were excised and treated with *C. granulosum* had statistically significant regression of their tumours when compared with animals whose melanomas were left *in situ* and treated with *C. granulosum*. There was no such regression of melanoma in animals treated with excision and injected with either *C. parvum* or BCG. Hence tumour excision was shown to be critical to regression only in *C. granulosum*-treated animals.

The one saline-treated animal whose primary tumour failed to regrow none the less had pulmonary metastases. The 12-day interval between initial tumour inoculation and subsequent excision apparently allowed ample time for metastases to have occurred (Paslin, 1973b).

Previous work had shown regression of this same hamster melanoma when injections of *C. granulosum* were begun with a palpable tumour diameter of 5 mm or less (Paslin *et al.*, 1974). The present work shows that regression did not occur when injections of *C. granulosum* were begun at a tumour diameter of 10 mm or more. It appears therefore that the critical tumour mass at which regression may occur in this system is less than 10 mm and greater than 5 mm.

The importance of tumour mass in determining regression of tumours is supported by evidence in other tumour systems. In mice bearing methylcholanthrene-induced tumours, tumour-specific immunity was measured by colony inhibition technique. A significant level of tumour-specific immunity was observed before palpable tumours developed. The level of immunity increased slightly as the tumours became palpable, then decreased and finally disappeared completely when the tumours were greater than 10% of total body weight. Resection rapidly increased the level of immunity. In the same study, the response of both T and B cells to mitogens decreased progressively with increasing tumour size, but returned to normal levels after resection (Whitney, Levy & Smith, 1974). In addition, other workers have shown that donor spleen cells from animals with small tumours are more effective inhibitors of tumour growth in syngeneic animals than donor spleen cells from animals with large tumours (Deckers, 1973).

BCG was ineffective in this hamster–melanoma system. In this regard, a study by Raynaud *et al.* (1972) compared selected immunogenic properties of *C. granulosum* and BCG. Their study showed that *C. granulosum* was more effective than BCG in stimulating carbon clearance and in enhancing antibody production. It may also be germane that corynebacterium macrophage chemotactic factor acts directly on macrophages. This differs from mycobacterial macrophage chemotactic factor which acts on macrophages via complement or a similar plasma enzyme system (Wilkinson, O'Neill & Wapshaw, 1973).

The difference in response of these hamster melanomas to *C. granulosum* and to *C. parvum* might be similarly explained by previously shown immunological differences among the species and strains of corynebacteria (Wilkinson *et al.*, 1973; O'Neill, Henderson & White, 1973; Adlam & Scott, 1972). Parameters studied by O'Neill *et al.* included carbon particle clearance, spleen and lymph node weight, antibody production and elaboration of a macrophage chemotactic factor. It was found that strains of *C. parvum* were generally more effective immune stimulants than the one strain of *C. granulosum* tested (O'Neill *et al.*, 1973). However, comparison of O'Neill's materials with ours is difficult, because a firm and satisfactory classification of the anaerobic corynebacteria has yet to be worked out. Species of corynebacteria bearing like names may represent different strains whose immunogenicity differ.

It cannot here be answered whether the *C. granulosum* in the animals whose melanomas were excised prevented the occurrence of pulmonary metastases or caused their regression once they had already occurred. The latter event is more likely, for it has been shown in this hamster–melanoma system that the capacity to metastasize is present by day 3 after tumour inoculation (Warren, 1966) and it has been shown that metastases have already occurred by week 3 after inoculation (Paslin, 1973b). Notwithstanding this evidence, work is in progress specifically to prove or disprove the occurrence and regression of metastases rather than the prevention of their occurrence.

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REFERENCES

ADLAM, C. & SCOTT, M.T. (1972) Lympho-reticular stimulatory properties of *Corynebacterium parvum* and related bacteria. *Journal of Medical Microbiology*, **6**, 261.

DECKERS, P.J. (1973) The effect of tumor size in concommitant tumor immunity. *Cancer Research*, **33**, 33.

O'NEILL, G.J., HENDERSON, D.C. & WHITE, R.G. (1973) The role of anaerobic coryneforms in specific and non-specific immunological reactions. I. Effect in particle clearance and humoral and cell mediated immunological responses. *Immunology*, **24**, 977.

PASLIN, D. (1973a) The effects of depigmenting agents in the growth of a transplantable hamster melanoma. *Acta dermato-venerologica*, **53**, 119.

PASLIN, D. (1973b) The effects of biopsy on the incidence of metastases in hamsters bearing malignant melanoma. *Journal of Investigative Dermatology*, **61**, 33.

PASLIN, D., DIMITROV, N.V. & HEATON, C. (1974) Regression of a transplantable hamster melanoma by intra-lesional injections of corynebacterium granulosum. *Journal of National Cancer Institute*, **52**, 571.

RAYNAUD, M., KOUZNETZOVA, B., BIZZINI, B. & CHERMANN, J.C. (1972) Etude de l'effet immunostimulant de diverses espèces de corynebacteries anaerobes et de leurs fractions. *Annales Institut Pasteur*, **122**, 695.

WARREN, B.A. (1966) The ultrastructure of capillary sprouts induced by melanoma transplants in golden hamsters. *Journal of the Royal Microscopical Society*, **86**, 177.

WHITNEY, R.B., LEVY, J.G. & SMITH, A.G. (1974) Influence of tumor size and surgical reaction in cell-mediated immunity in mice. *Journal of the National Cancer Institute*, **53**, 111.

WILKINSON, P.C., O'NEILL, G.J. & WAPSHAW, K.G. (1973) Role of anaerobic coryneforms in specific and non-specific immunological reactions. II. Production of a chemotactic factor specific for macrophages. *Immunology*, **24**, 997.

British Journal of Dermatology (1976) **94**, 645.

Lichen verrucosus et reticularis of Kaposi (porokeratosis striata of Nékam): a manifestation of acquired adult toxoplasmosis

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SUMMARY

Three young adults are described with features of lichen verrucosus et reticularis of Kaposi (porokeratosis of Nékam). Serological evidence of toxoplasmosis (active or recent infection) was found in all three. Marked improvement on appropriate treatment occurred in the one patient who presented evidence of systemic toxoplasmosis.

In 1895 Kaposi described a 27-year-old female with a linear, warty, lichenoid eruption arranged predominantly along the limbs to which he gave the name of lichen ruber verrucosus et reticularis. Kaposi had also previously, in 1886, described another patient with similar clinical features under the title of lichen ruber moniliformis. Nékam reviewed both these cases in 1938. The term porokeratosis striata lichenoides was introduced by him as he considered it to be a more suitable description than lichen ruber verrucosus et reticularis. The nature of these striking skin lesions has remained obscure. Recently, the description of toxoplasmosis with associated lichen planus-like eruptions (Andreev, Angelov & Zlatkov, 1969) and Justus's comprehensive review of cutaneous manifestations in toxoplasmosis (1972), led us to examine three patients with skin lesions very similar to Kaposi's original patients for possible toxoplasmosis.

CASE REPORTS

Case 1

A 26-year-old black woman from the Eastern Transvaal gave an 18-month history of an itching eruption of acute onset on the face and limbs. This was associated with mouth ulceration, followed by eye trouble leading gradually to almost complete blindness. She also complained of marked weight loss, painful joints, progressive cough with hoarseness and amenorrhoea for 9 months. She was childless with a history of three spontaneous abortions.

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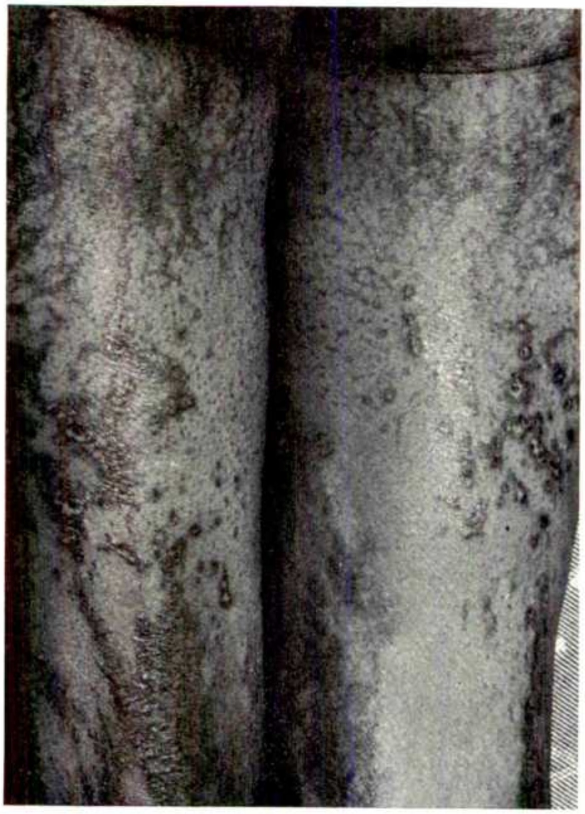

FIGURE 1. Case 1. Linear and striate areas on posterior aspects of thighs.

Examination revealed a striking picture. Arranged predominantly longitudinally along the limbs, was a violaceous, lichenoid eruption in which striate and interlaced ridges were most marked around the flexures of the knees and elbows and posterior aspects of the thighs (Fig. 1). Interspersed were more hyperkeratotic, discrete papules and there were in addition large purpuric plaques and a violaceous, thickened area with a haemangiomatous appearance extending from the right big toe (Fig. 2) along the inner calf. The palms and soles were markedly hyperkeratotic and the nails were dystrophic, thickened and ridged with flexion contractures of the fingers (Fig. 3). The face was oedematous with erythema, scaling and multiple small papules. Numerous discrete painful ulcers were present on the tongue and buccal mucosa. There were multiple reticulated areas of pigmentation on the trunk and thighs with associated scaliness. All the lymph glands were palpable, being small, firm, discrete and mobile in the neck but much larger in the axillae and groins where a marked 'boggy' periadenitis was present (Fig. 4). These swellings around the glands had the consistency of adult female breast tissue. Examination of the eyes revealed a bilateral kerato-conjunctivitis with deep vascularization of the cornea. Her temperature on admission was 39°C and persisted as a continuous fever thereafter.

Investigations

Skin biopsy. Parakeratotic and acanthotic epidermis, with a chronic inflammatory cell infiltrate in the upper dermis associated with marked pigment incontinence and isolated colloid bodies, suggested lichen planus. In the deeper vessels a vasculitis of the leukocytoclastic type was demonstrated.

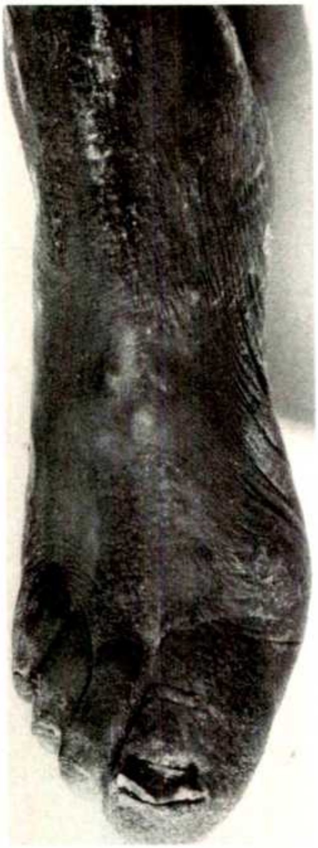

FIGURE 2. Case 1. Angiomatous thickening of right big toe and hyperkeratotic lesions on ankle.

Lymph node biopsy. The cortical, paracortical and medullary areas appeared normal and there was no convincing evidence of epithelioid cell granulomas. No organisms were demonstrated by Giemsa staining.

Bronchoscopy and bronchial biopsy. Thickening of the mucosa of the pharynx and larynx was associated with small white stippling on the surface. The vocal cords were also thickened with similar white dots. Histological examination showed chronic inflammatory changes with no demonstrable organisms.

Tests for toxoplasmosis. *Toxoplasma gondii* complement fixation test was positive in a titre of 1:32 and the Sabin Feldman dye test gave an original titre of 1:1024. The anti-toxoplasmosis fluorescent antibody test was positive on three occasions. These tests indicate active or recently active toxoplasmosis. Cultures were unfortunately not attempted.

Treatment and progress. Numerous diagnoses including histoplasmosis, sarcoidosis, syphilis, tuberculosis, reticulosis, Behçet's disease, warty lichen planus, pityriasis rubra pilaris, erythema multiforme, angiomatosis, lichen ruber moniliformis were considered. A long time elapsed before the diagnosis of toxoplasmosis was entertained. Numerous treatments were tried, aimed at various

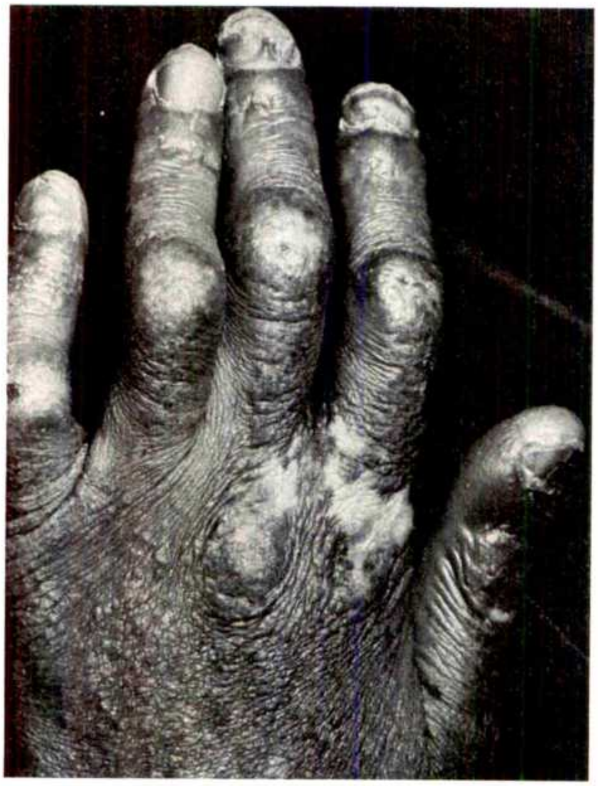

FIGURE 3. Case 1. Flexion contractures of fingers, nail dystrophy and multiple papules on hand.

possible but unproven diagnoses. Apart from some stabilization of the pyrexia on anti-tuberculosis therapy no other improvement was noted on any of the other treatments, which included tetracycline, erythromycin, penicillin and prednisone. Improvement within 72 h was seen only after sulphadiazine and pyrimethamine were given as for toxoplasmosis. The temperature became normal, the oedema around the glands in the axillae and groins subsided, the patient gained weight and felt much better. Her vision improved. The swelling and erythema of the face subsided and healing of the mucosal ulcers and flattening of the papular lesions took place. The more keratotic areas around the knees and elbows remained virtually unchanged.

The further management was not entirely straightforward, however. After 10 days' treatment pyrimethamine was stopped while sulphadiazine was continued. A second course of pyrimethamine was begun 10 days later and the temperature immediately rose. Treatment was then stopped after 21 days in the belief that the patient was suffering from a drug fever. However, the temperature rose further to 40·5°C and only became normal after steroids were given.

Case 2

A 43-year-old black male from the Transvaal presented with skin lesions which had started with asymptomatic patches on the backs of his hands 6 years previously. This rash had spread to involve the elbow regions as well as the rest of the arms, buttocks and thighs. On examination the lesions consisted of linear, hyperkeratotic papules most marked around the elbow flexures with scattered similar papules on the thighs and buttocks. Multiple lichenoid papules were also present on the backs

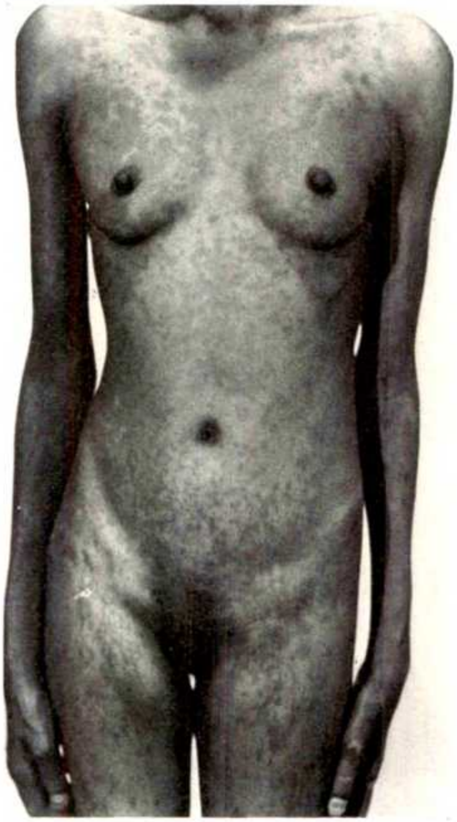

FIGURE 4. Case 1. Marked swelling around the glands in the groin. Multiple pigmented areas on trunk and thighs.

of the hands with some individual papules showing a depressed centre giving a porokeratotic appearance (Fig. 5). The rest of the skin and the nails were normal. The patient was otherwise well, with no signs of ocular or glandular involvement.

Investigations

Skin biopsy. Thickened parakeratotic epidermis with a diffuse lymphohistiocytic infiltrate in the upper dermis was present, disrupting the basement membrane zone (Fig. 6). Numerous colloid bodies were present together with pigment incontinence. In the deeper dermis there was a marked perivascular inflammatory infiltrate which also involved the vessel walls (Fig. 7). Mild haemorrhage was also noted.

Serological tests for toxoplasmosis. Positive fluorescent antibody tests were found on two occasions.

Treatment and progress. There was no improvement of the skin lesions with two courses of sulphadiazine and pyrimethamine.

Case 3

A 30-year-old black female from Malawi presented with a 2-year history of an itching eruption. The

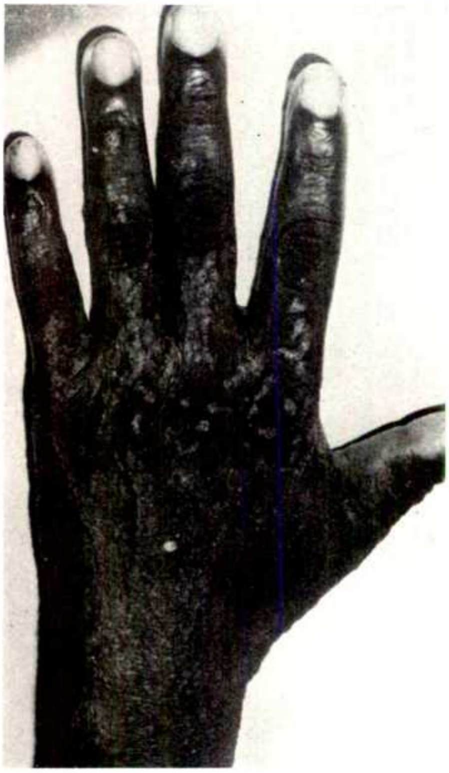

FIGURE 5. Case 2. Lichenoid papules on hand with depressed centres giving a porokeratotic appearance.

skin of the elbow flexures showed multiple, warty, infiltrated papules arranged longitudinally (Fig. 8), with scattered papules on the face and discrete lesions on the forearms, dorsa of hands and lateral aspects of the trunk and legs. Numerous superficial buccal ulcers were also present as well as small white papules on the soft palate. There was no lymphadenopathy and the patient was otherwise well.

Investigations

Histology. Hyperplastic epidermis was present, with a diffuse lymphohistiocytic infiltrate in the upper dermis in which region there was marked pigment incontinence. The deeper dermis showed a perivascular and peri-adnexal infiltrate.

Tests for toxoplasmosis. Toxoplasma indirect fluorescent antibody test showed a positive reaction up to a dilution of 1:80.

Progress and treatment. A 6-week course of cotrimoxazole resulted in considerable improvement in the skin lesions of the face and trunk with no flattening in the more warty lesions on the elbow flexures. Unfortunately the patient did not return for further treatment or follow-up.

DISCUSSION

Kaposi (1895) and Nékam (1938) described a patient with a patterned eruption on the limbs, multiple

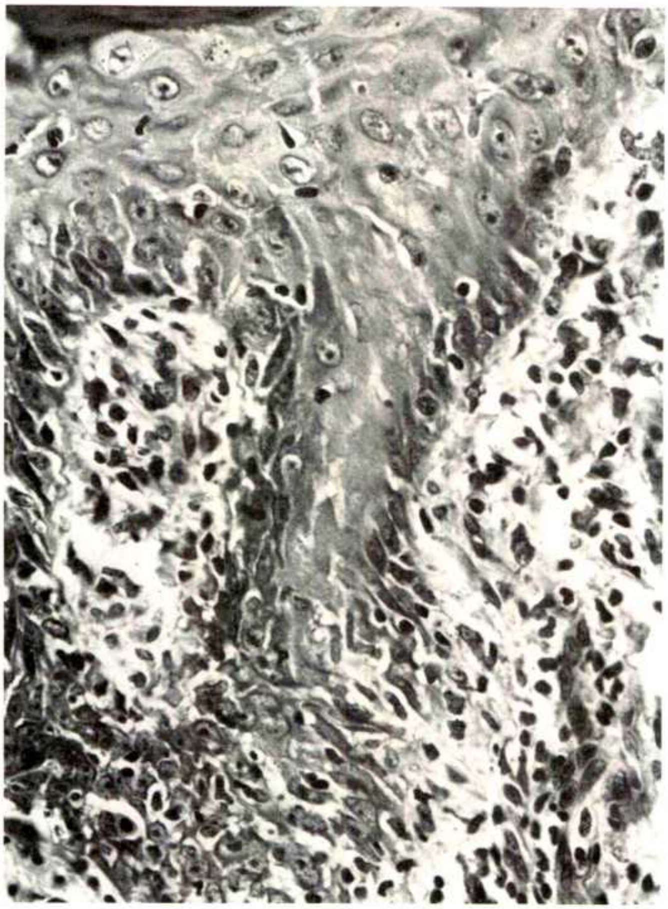

FIGURE 6. Case 2. Lymphohistiocytic infiltrate in upper dermis disrupting basement membrane zone (H & E, × 400).

keratotic papules down the trunk, hyperkeratotic palms and soles with associated nail changes. In addition their patient had mouth ulcers, enlarged glands and later developed eye changes leading to blindness. She also had a history of eight pregnancies with two ending in spontaneous abortions. Histological examination showed, in addition to lichen planus-like changes, a marked vasculitis which Nékam thought was the basic pathological feature of the disease. This case, which was called lichen ruber verrucosus et reticularis by Kaposi and porokeratosis striata lichenoides by Nékam, bears an exact resemblance to our first case.

Kaposi's second patient (1886), which he called lichen ruber monileformis, was also reviewed by Nékam who considered it to be lichen planus with a tendency to keloid formation and distinct from lichen ruber verrucosus et reticularis. Kaposi's two patients had similar skin changes alone but one (1895) had systemic involvement as well. Several cases with similar skin changes have subsequently been described. In the case described by Wise & Rein (1936) there was vasculitis, and a patient described by McFarland (1947) had a histological picture suggesting periarteritis nodosa. Miller's case (1945), a 38-year-old woman, showed a red scaly eruption on the face, violaceous, hyperkeratotic linear lesions on the arms and discrete conical papules on the buttocks. Her first pregnancy ended in a stillbirth.

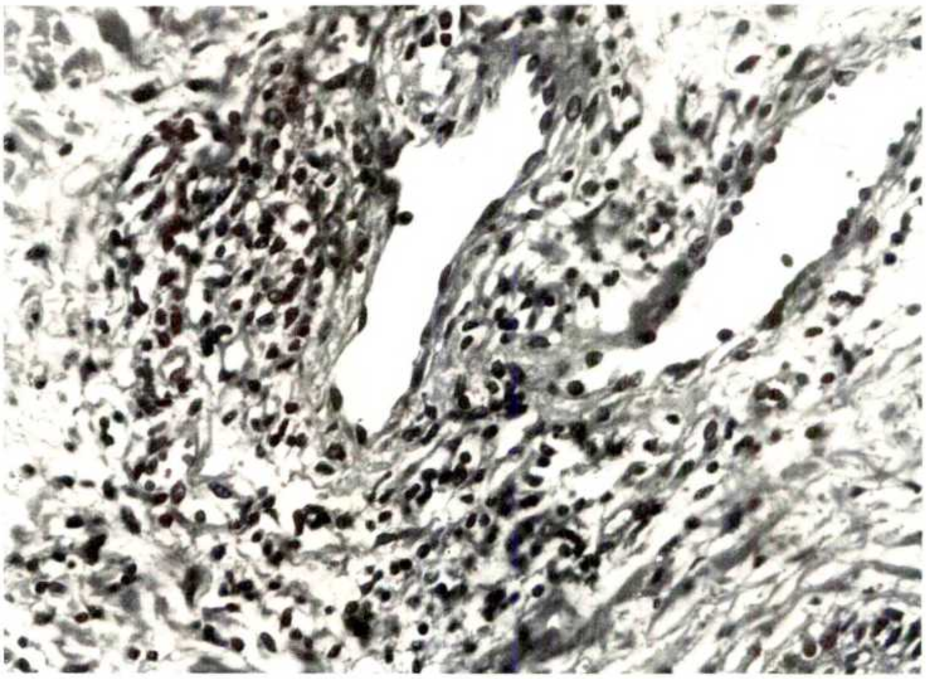

FIGURE 7. Case 2. Perivascular infiltrate involving vessel walls in deeper dermis (H & E, × 400).

Pinkerton & Weinmann (1940) and Pinkerton & Henderson (1941) were the first to demonstrate skin lesions in patients with toxoplasmosis. Various descriptions were given to the lesions in these patients ranging from nodules to macular and papular eruptions. Parasites were not found in the skin biopsies. Koravitzkii *et al.* in 1964 described seven cases with lymph gland enlargement as well as bluish, purplish livedo-like changes on the limbs and arms.

The association of toxoplasmosis with lichen verrucosus et reticularis (porokeratosis striata of Nékam) has, as far as we can ascertain, not yet been described. Justus's statement that there is 'no absolute pathognomonic macroscopic or microscopic picture of the skin involvement of toxoplasmosis (acquired adult)' is borne out by the wide variety of cases described in which skin changes are prominent.

Braun, Petter & Wildführ (1968) described a case with acquired toxoplasmosis. The patient, a 34-year-old female, presented with fever, red eyes, lymphadenopathy, hepatitis and widespread follicular scaly papules with thick fissured palms and soles and keratotic nails resembling pityriasis rubra pilaris. *Toxoplasma gondii* was isolated from skin cultures. The patient, while on anti-toxoplasmosis treatment, developed a severe allergic fever which was checked only by steroids. In our first patient the same problem arose.

Toxoplasmosis is an extremely common disease, which is thought to account for 15% of cases of otherwise unexplained lymphadenopathy in some parts of the world (W.H.O. Bulletin, 1969). The Public Health Laboratory Services in the United Kingdom and the Republic of Ireland reported 1071 cases of toxoplasmosis in 1971 and 941 cases in 1972 (*Epidemiology*, 1974). Most (55%) of the cases had eye diseases such as uveitis or choroiditis, with lymphadenopathy or unexplained pyrexia in 27%. Infestation *in utero* has been shown to cause abortion or stillbirth, mental retardation, hydrocephalus, hepatosplenomegaly and uveitis. Acquired infestation from raw or undercooked meat

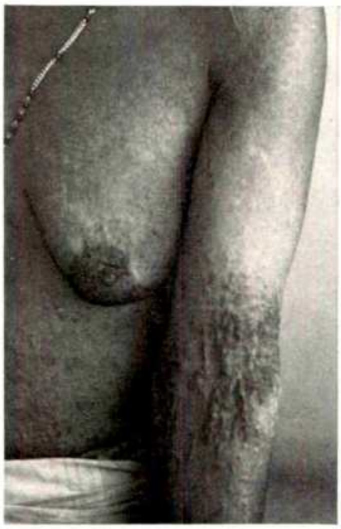

FIGURE 8. Case 3. Warty linear thickenings on elbow flexure with multiple papules on trunk.

is well recognized but the importance of the domestic cat as a reservoir of *Toxoplasma gondii* is now becoming evident (Markus, 1973). A recent survey from the Transvaal, South Africa, using the toxoplasma indirect fluorescent antibody test gave a 37% positive titre at a dilution of 1:16 or higher from 805 routine samples of human sera (Mason, Jacobs & Fripp, 1974). These authors stress the importance of contact with felines in the epidemiology of the disease.

Evidence that the skin lesions in our patients were caused by toxoplasmosis is based on positive serological tests which suggested recent or active infestation. Proof of this association would be obtained by the isolation of the organisms from skin and lymph gland inoculates into mouse peritoneal cavity. It was not performed in our patients. It may also be argued, with the high known incidence of toxoplasmosis in the South African population, that our findings are coincidental. However, the dramatic clinical improvement with appropriate treatment in case 1 in which all other regimes had failed, strongly suggested that an active toxoplasmosis infection was responsible for the systemic signs. Kaposi and Nékam's case had a systemic disease with severe eye changes leading to blindness, lymphadenopathy and mouth ulceration. In addition the skin lesions showed histological evidence of a vasculitis. Our first case is practically identical in every respect. The fact that our other two patients also had serological tests indicating active or recent toxoplasmosis suggests that skin lesions described as lichen verrucosus et reticularis of Kaposi (porokeratosis of Nékam) may be due to toxoplasmosis in a less virulent form.

ADDENDUM

After completion of our study, it was pointed out to us that a closely similar systemic illness had been described recently under a new name, Lichenoid Tri-Keratosis, by Piñol Aguadé, de Asprer & Ferrando (1974). Among other recent reports with similar findings, though varying in severity, the following should be noted: Chapman (1971) and Degos *et al.* (1974). In none of these had the possibility of toxoplasmosis been considered.

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REFERENCES

ANDREEV, V.C., ANGELOV, N. & ZLATKOV, N.B. (1969) Skin manifestations in toxoplasmosis. *Archives of Dermatology*, **100**, 196.

BRAUN, W., PETTER, O. & WILDFÜHR, G. (1968) Akute erworbene Toxoplasmose unter dem Bild einer Pityriasis rubra pilaris. *Archiv für klinische und experimentelle Dermatologie*, **232**, 295.

CHAPMAN, R.S. (1971) Lichen verrucosus and reticularis. *Dermatologica*, **142**, 363.

DEGOS, R., LABOUCHE, F., CIVATTE, J., TOURAINE, R. & BALOUET, G. (1974) Kératose lichénoide striée. *Annales de Dermatologie et de Syphiligraphie, Paris*, **101**, 391.

EPIDEMIOLOGY—TOXOPLASMOSIS (1974) In: News and notes. *British Medical Journal*, **iii**, 695.

JUSTUS, J. (1972) Cutaneous Manifestations of Toxoplasmosis. In: *Current Problems in Dermatology* (Ed. by J.W.H.Mali), Vol. 4, p. 24. Karger, Basel.

KAPOSI, M. (1886) Lichen ruber monileformis-Korallen-schnurartiger Lichen ruber. *Archiv für Dermatologie und Syphilis*, **18**, 571.

KAPOSI, M. (1895) Lichen ruber acuminatus und Lichen ruber planus. *Archiv für Dermatologie und Syphilis*, **31**, 1.

KOROVITZKII, L.K., BORSOV, M.V., LOBANOVSKY, G.I. & BOGDANUK, L.S. (1964) Skin lesions in toxoplasmosis (in Russian). *Vestnik dermatologii i venerologii*, **38**, 28.

MARKUS, M.B. (1973) Symptoms, transmission, prevention and treatment of toxoplasmosis. *South African Medical Journal*, **47**, 1588.

MASON, P.R., JACOBS, M.R. & FRIPP, P.J. (1974) Serological Survey of toxoplasmosis in the Transvaal. *South African Medical Journal*, **48**, 1707.

MCFARLAND, A.R. (1947) Morbus moniliformis lichenoides, variant types. *Archives of Dermatology*, **55**, 674.

MILLER, H.E. (1945) Lichen verrucosus et reticularis (Kaposi); porokeratosis striata (Nékam); morbus moniliformis lichenoides (Wise and Rein). *Archives of Dermatology*, **52**, 129.

NÉKAM, L. (1938) Sur la question du lichen moniliforme. *Presse Médicale*, **51**, 1000.

PINKERTON, H. & HENDERSON, R.G. (1941) Adult toxoplasmosis: a previously unrecognized disease entity simulating the typhus-spotted fever group. *Journal of the American Medical Association*, **116**, 807.

PINKERTON, H. & WEINMAN, D. (1940) Toxoplasma infection in man. *Archives of Pathology*, **30**, 374.

PINOL AGUADÉ, J., DE ASPRER, J. & FERRANDO, J. (1974) Lichenoid tri-keratosis. *Dermatologica*, **148**, 179.

WISE, F. & REIN, C.R. (1936) Lichen ruber moniliformis (morbus moniliformis lichenoides). Report of a case and description of a hitherto unrecorded histologic structure. *Archives of Dermatology*, **34**, 830.

WORLD HEALTH ORGANIZATION TECHNICAL REPORT SERIES (1969) *Toxoplasmosis, no. 431*. Report of a WHO meeting of Investigators, Geneva.

British Journal of Dermatology (1976) **94**, 655.

Accidental kidney biopsies in psoriasis

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SUMMARY

Eight accidental kidney biopsies in psoriatics were obtained together with 429 liver biopsies. None of the biopsies showed signs of renal damage. All patients had severe psoriasis, six had been treated with methotrexate for 1–2·6 years, receiving total dosages from 660 to 1757 g.

It is well known that 50–90% of each dose of methotrexate normally is excreted in the urine (Calabres & Parks, 1970) and that methotrexate should be given rarely to patients with impaired renal function. Although renal toxicity of methotrexate has only seldom been reported (Baker, 1970), death attributable to methotrexate-induced renal failure has been mentioned at least twice (Ryan & Vickers, 1966; McKenzie & Aitken, 1967). We therefore thought it of interest to report the normal histology in eight accidental kidney biopsies obtained as additional material during liver biopsies in psoriatics.

MATERIALS AND METHODS

Eight accidental kidney biopsies were obtained together with 429 liver biopsies. Six of these were in psoriatics treated with methotrexate from 1 to 2·6 years. Two biopsies were in psoriatics considered for treatment with methotrexate. All had a normal serum creatinine. The age and sex of the patients together with the total accumulative methotrexate dosage appear in Table 1.

TABLE 1. Serum creatinine and total cumulative dosage of methotrexate in psoriatics with accidental kidney biopsies

Patient	Sex/age	Dosage g	Treatment (years)	Serum creatinine (mg/100 ml)
1	F/30	—	—	0·9
2	F/65	—	—	0·8
3	F/65	660	1	0·8
4	M/65	720	1	1·3
5	M/46	780	1	1·1
6	F/72	950	1·5	1·0
7	F/32	1120	1·5	0·8
8	F/33	1757	2·6	0·9

All biopsies were obtained by the Menghini technique (Menghini, 1958) using a 70 × 1·9 mm needle. In no case did the patients show signs of damage due to this complication. Post-biopsy urine examination gave normal results.

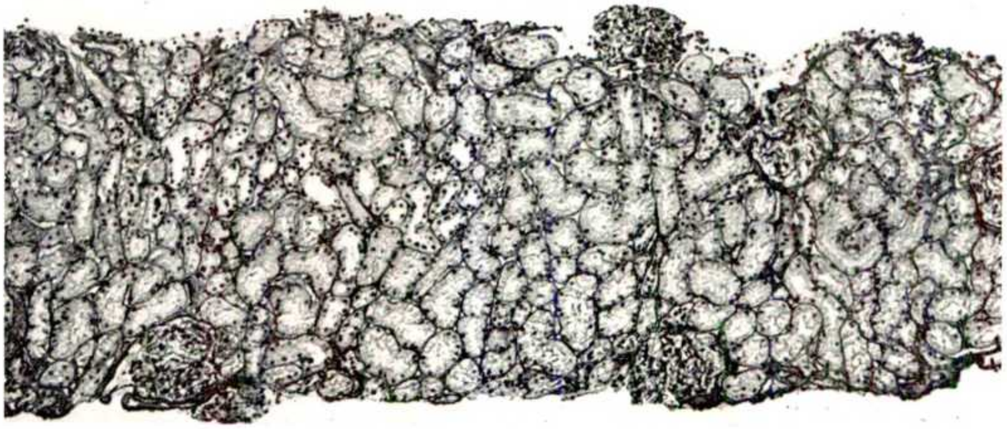

FIGURE 1. Normal kidney biopsy from methotrexate treated psoriatic (H & E, × 30).

TABLE 2. Liver biopsies in eight patients, from which accidental kidney biopsies were obtained. The figures are gradings, from 1 to 4 representing grade of histological abnormality. Grade 1 represents no abnormality.

Patient	Sex/age	Steatosis	Nuclear variability	Periportal inflammation	Focal necrosis	Cholestasis	Fibrosis
1	F/30	1	1	1	1	1	1
2	F/65	2	1	1	1	1	1
3	F/65	2	2	1	1	1	1
4	M/65	2	2	1	1	1	1
5	M/46	3	2	1	1	1	1
6	F/72	2	1	1	1	1	1
7	F/32	1	1	1	1	1	1
8	F/33	4	4	2	2	1	2

RESULTS

All kidney biopsies were normal and showed no signs indicating kidney damage. Fig. 1 shows one of these biopsies. Results of liver biopsies in the same patients are shown in Table 2. Most biopsies showed mild to moderate steatosis. One liver biopsy (Patient 8) showed severe steatosis and nuclear variability, mild periportal inflammation, focal necrosis and fibrosis together with signs of beginning cirrhosis. This patient had 2 years earlier shown an almost normal liver biopsy.

DISCUSSION

All renal biopsies were normal, including the biopsy from a patient with methotrexate induced liver cirrhosis.

Although in cancer treatment renal damage does not seem uncommon, methotrexate almost never seems to cause renal toxicity in psoriasis. This obviously seems to be a question of dose. However, severe psoriasis seems to be a more generalized disease than is normally accepted and has been shown to produce lesions in several other organs than the skin (Wright, 1959; Zachariae & Søgaard, 1973; Nielsen & Zachariae, 1973; Grunnet, Nyfors & Brogaard Hansen, 1975). The results of our biopsies should be viewed in this perspective.

Our results do not indicate methotrexate nephrotoxicity, neither have they indicated that severe psoriasis in itself produces renal damage. We are well aware, however, that more general conclusions cannot be drawn from these results due to the limited number of biopsies.

REFERENCES

BAKER, H. (1970) Some hazards of methotrexate treatment of psoriasis. *Transactions of the St John's Hospital Dermatological Society*, **56**, 111.

CALABRES, I. & PARKS, R. (1970) Chemotherapy of neoplastic diseases. In: *The Pharmacological Basis of Therapeutics* (Ed. by L.Goodman and E.Gillman), 4th edn. Macmillan, New York.

GRUNNET, E., NYFORS, A. & BROGAARD HANSEN, K. (1976) Studies on human semen in severe psoriasis. To be published.

MCKENZIE, A. & AITKEN, C. (1967) Psoriasis and folic acid antagonists. *British Journal of Dermatology*, **79**, 122.

MENGHINI, G. (1958) One-second needle biopsy of liver. *Gastroenterology*, **35**, 190.

NIELSEN, J. & ZACHARIAE, H. (1973) Chromosome aberrations in severe psoriasis. *Acta dermato-venereologica*, **53**, 192.

RYAN, T. & VICKERS, H. (1966) Psoriasis and folic acid antagonists. *British Journal of Dermatology*, **78**, 612.

WRIGHT, V. (1959) Psoriatic arthritis. *Archives of Dermatology*, **80**, 65.

ZACHARIAE, H. & SØGAARD, H. (1973) Liver biopsy in psoriasis. A controlled study. *Dermatologica*, **146**, 149.

British Journal of Dermatology (1976) **94**, 659.

Failure to produce warts on human skin grafts on 'nude' mice

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SUMMARY

Split-thickness human skin was exposed to a suspension of human papilloma virus containing 10^{11}–10^{12} particles/ml, by immersion for varying lengths of time. It was then grafted onto the backs of congenitally athymic 'nude' mice. All grafts were accepted, but no warts developed during an observation period of up to 9 weeks.

Research into the prevention and possible cure of warts has been hampered by the lack of an *in vitro* assay system. Attempts to grow the virus in tissue culture have been and are continuing to be relatively unsuccessful (Cubie, 1972; Butel, 1972; Cubie, 1974; Abramson, 1975). It was therefore felt that an entirely different approach making use of a specialized animal system might yield more positive results.

The hairless mouse mutant 'nude' (Flanaghan, 1966) was chosen because it is congenitally athymic (Pantelouris, 1968). In the past, there have been problems in the use of 'nude' mice because of their low fertility and poor survival (De Sousa, Parrott & Pantelouris, 1969; Wortis, Nealsen & Owen, 1971), but these can be alleviated by the use of specific pathogen-free (SPF) conditions for both breeding and rearing (Giovanella & Stehlin, 1973; Rygaard & Friis, 1974). The failure of cell-mediated immune mechanisms is reflected in the complete acceptance of skin grafts from many sources, not only from rodents (Rygaard, 1969; Pantelouris, 1971) and humans (Cubie, 1972; Reed & Manning, 1973; Rygaard, 1974), but also from animals of different phyla (Manning, Reed & Shaffer, 1973).

Thus, 'nude' mice grafted with human skin provided a system closer to that in which natural infection occurs than was possible under tissue culture conditions. In addition, more information about the acceptance and healing of split-thickness human skin grafts on 'nude' mice was obtained.

MATERIALS AND METHODS

Mice. Female 'nude' mice, which had been born and reared under SPF conditions from homozygous male 'nudes' and heterozygous haired sisters, were received at the age of 5–6 weeks. They were housed in a specially constructed cabinet into which room air, filtered of particles greater than 5 μm in size, was passed. The cabinet itself provided a barrier against airborne contamination and it was further isolated from the conventional animal house by being kept in a separate room with restricted access. Protective clothing was worn by all workers entering the room. Mouse boxes were autoclaved complete with sawdust bedding; water and waterbottles were also autoclaved and the mice were provided with a commercially autoclaved small animal diet.

Infecting and grafting of human skin. Thirty-four 'nude' mice between 5 and 8 weeks old were grafted under pentobarbitone anaesthesia, using the technique of Billingham & Medawar (1951) with split-thickness human skin from the thigh or arm. The donor skin was used as fresh as possible and generally within 6 days. It was stored at 4°C in saline-moistened gauze under sterile conditions. That viability was maintained under these conditions was shown by the acceptance of a graft of 27-day-old skin.

The grafts were approximately 0·5 cm^2 in size and cut to fit the graft bed. They were either sutured at the corners or held in place with a tissue adhesive spray. Jackets consisting of framycetin gauze, non-allergic surgical tape and waterproof adhesive tape were wound round the animals' bodies and left for 7 days. Eighty per cent of grafted mice survived until the jackets were removed.

In the majority of cases, human skin was infected prior to grafting by immersion in a suspension of semi-purified human papilloma virus containing 10^{11}–10^{12} particles per ml (Cubie, 1972) for various lengths of time ranging from 10 to 90 min. Scarification of accepted grafts was also tried, but the delay between grafting and subsequent introduction of virus made this technique less useful.

Grafted mice were examined twice weekly for signs of lesions until the mice became moribund. They were then killed by cervical dislocation. The graft area was removed immediately and immersed in Karnowsky's fixative for electron microscopy, or formalin or formal calcium for light histology.

Histological methods. Fixation of skin for electron microscopy was carried out in half-strength Karnowsky's fluid (Karnowsky, 1967) for 5 h at room temperature. The material was post-fixed in osmium tetroxide and embedded in araldite resin by standard electron microscopy techniques. Thick sections were first examined and then appropriate thin sections were cut on an LKB ultramicrotome. The sections were stained with uranyl acetate and lead citrate and viewed on a Hitachi HU12A electron microscope at 50 KV.

Fixed material for light histology was embedded in paraffin wax and sections were stained with haematoxylin and eosin according to standard procedures.

RESULTS

Macroscopic appearance of grafts. Immediately after removal of the jackets, the grafts appeared white and supple, but tended to dry out slightly during the next 48 h. The edges of the grafts usually healed within 2–3 weeks and all were completely accepted, although small patches of graft loss were occasionally observed. There was no difference in general appearance between control and infected grafts (Table 1). By 10–14 days, some grafts developed a thick necrotic scab in the centre with healthy pink skin round it, and this took a further week or two to heal. This shedding of the old cuticle has been described as a 'ghost graft'. They occurred more often here when the mouse muscle layer, the panniculosus carnosus, had been cut or removed. An intact panniculosus enabled vascularization and healing of the graft to take place more readily.

Twelve mice developed small 'nodules' within the grafted area about 3 weeks after grafting. These were usually pinky-brown, raised and firm to touch (Fig. 1). Eleven of these mice had received infected human skin, but the remaining mouse carried an uninfected control graft.

TABLE 1. Macroscopic changes in human skin grafts on 'nude' mice following exposure to human papilloma virus

Initial treatment of graft		Normal grafts: No. of grafts with healthy appearance	Normal grafts: No. of grafts developing 'ghost grafts'	No. of grafts developing 'nodules'	Totals
Immersion in virus for:	10–30 min	5	2	4	11
	30–60 min	1	1	3	5
	60–90 min	0	1	4	5
	overnight	2	1	0	3
Scarification		2	2	0	4
Uninfected controls		0	5	1	6
Totals		10	12	12	34

Microscopic appearance of grafts. By 8 days after grafting it could be seen histologically that the grafts had been accepted. The dermis of the graft appeared very fibrocellular and revascularization had begun. The epidermis showed a flat basal layer and exhibited slight hypergranulosis and hyperkeratosis. From 3 weeks, the grafts were well established with a vascular dermis, distinct rete pegs and slight acanthosis. This can be seen in Fig. 2 in which the human/mouse skin junction is also visible.

In two grafts of 47 and 52 days in which macroscopic nodules developed, epidermal thickening was pronounced, orthokeratosis was evident, and occasional small keratinized intra-epidermal cysts were noted. There was no evidence of the distinctive parakeratosis found in wart lesions, and no vacuolization or eosinophilic inclusions were observed in cells of the granular layer.

A 16-day graft which had developed a necrotic scab appeared similar at the edges to the normal grafts, while below the crust the dermis contained mixed inflammatory cells of a chronic nature. Electron microscopically, a similar graft showed highly active epidermal cells below the crust indicating that regeneration and remodelling were occurring.

Six-day-old ungrafted skin was examined in the electron microscope before and after immersion in virus. Both pieces were similar and appeared very degenerate with dispersed nuclear chromatin and vacuolated, shrunken cells. In the superficial layers, keratohyalin granules tended to distort nuclear

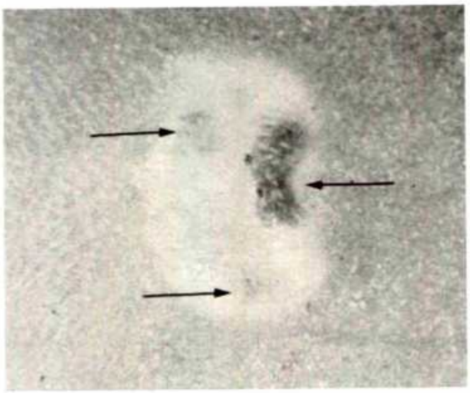

FIGURE 1. Thirty-day human skin graft on the back of a 'nude' mouse. The skin was infected with human papilloma virus prior to grafting and developed one large and two small 'nodules' (arrows).

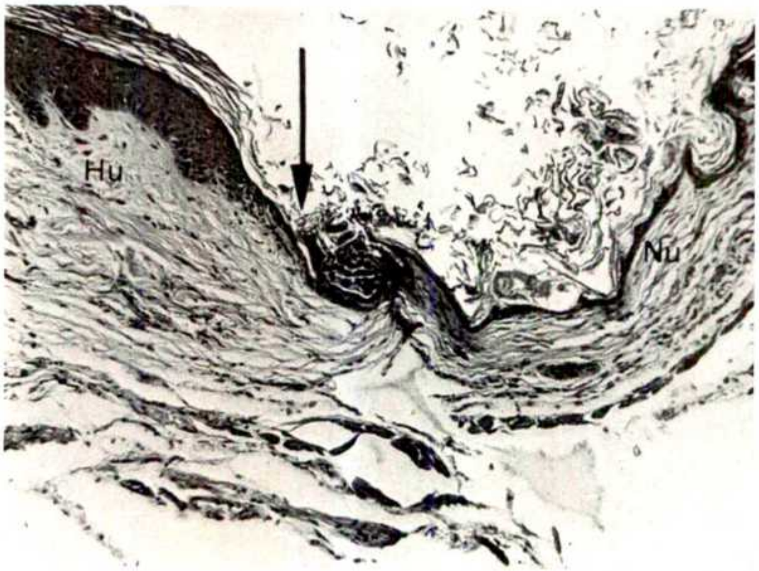

FIGURE 2. Fifty-two-day infected graft, with distinct rete pegs, acanthosis and hyperkeratosis. The human/mouse skin junction is also visible (arrow), with human skin (Hu) to the left and 'nude' mouse skin (Nu) to the right.

shape and lower down there was almost complete separation of the epidermis from the underlying dermis. Nevertheless, the same skin was used for two grafts which remained healthy until the deaths of the mice, 49 and 52 days later.

The ultrastructural appearance of grafted skin resembled normal skin, the usual dermal and epidermal elements being observed. In a few grafts, Langerhans cells appeared active and more numerous than expected, but quantitative studies were not carried out. Granules attached to their cell walls seemed also to be more common than in normal skin. In addition, intranuclear particles of approximately 35–45 nm in diameter were observed in keratinocytes of all the grafts examined (Fig. 3). They were round and regular in appearance, often surrounded by a small halo and usually occurred singly or in small groups of 3–5 particles. They were similar to particles of human papilloma virus in sections of plantar warts, particularly where the virus particles occurred individually rather than in clumps or inclusions (Fig. 3, inset). They were, however, also observed in the three control grafts examined and also, though to a lesser extent, in a skin biopsy from a healthy adult.

Survival of mice and grafts. The grafted mice lived for an average of 83·6 days with a range from 52 to 105 days, though only four mice lived for more than 100 days. The grafts themselves remained *in situ* for an average of 32·5 days with a range from 14–63 days. Grafts developing nodules were older than those which did not develop nodules (Table 2).

TABLE 2. Survival of 'nude' mice after grafting with human skin

	Grafts without nodules	Grafts developing nodules	Combined results
Survival range (days) after grafting	14–63	33–52	14–63
Average survival (days)	28·0	42·9	32·5

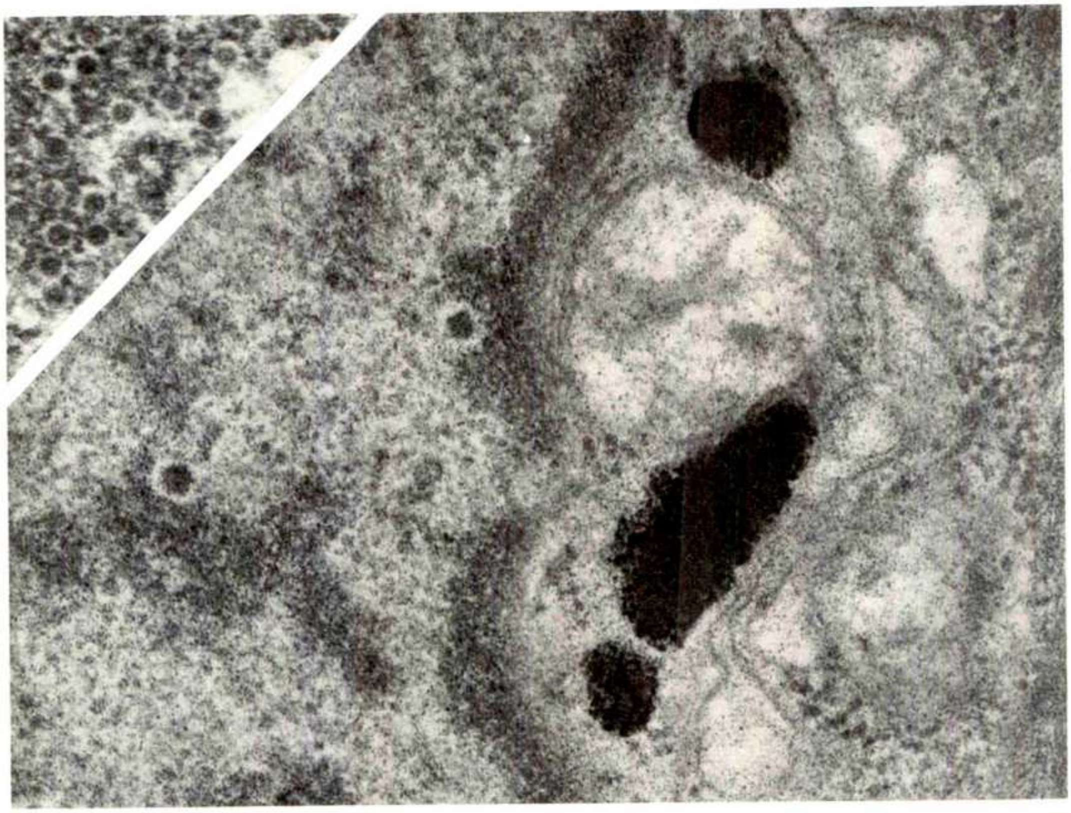

FIGURE 3. Two particles within the nucleus of a keratinocyte of a 30-day infected graft (same graft as in Fig. 1) (× 78,000).

Inset. Particles of human papilloma virus within the nucleolar matrix of a keratinocyte from a simple plantar wart (× 78,000).

DISCUSSION

The acceptance and survival of human skin grafts on 'nude' mice has already been reported (Reed & Manning, 1973; Rygaard, 1974), but no nodular changes like those described here were noted. Keratin cysts have, however, been regularly observed within human skin autografts applied to tangentially excised burns (Stone & Lawrence, 1973). These are generally caused by keratinization within areas of hyperplastic epidermal cells arising from hair shafts or residual sebaceous glands in the graft or its bed. Such cysts can take up to 6–9 months to resolve completely (Sutherland, 1975) either by intradermal rupture or by external discharge. A similar explanation seems possible for the nodules noted here, although none resolved during the observation period available. That the nodules could have been the result of successful infection with human papilloma virus seems unlikely since nodules developed in one graft which had no contact with virus. Furthermore, grafts which developed nodules had survived longer than those without, and this effect might have been observed more regularly if all grafts had survived for an equal length of time.

The intranuclear 'particles' observed in electron microscopical preparations of graft tissue are difficult to explain. Their presence in control grafts precludes virus particles even though the size range is appropriate. A possible explanation could be that they represent a latent mouse virus, activated by the grafting procedure and capable of infiltrating the graft. However, since ungrafted human skin appeared to contain similar, though certainly less distinct, particles, they may well represent a preparative artefact, or small pieces of condensed chromatin, with their observation here being due to specific searching for particles of this size.

Kreider & Breedis (1969) succeeded in infecting fetal rat skin by incubating the skin in a suspension of Shope papilloma virus, and it was felt that a similar technique might be useful with the corresponding human virus. Failure to transmit infectivity might be explained by a low infectivity in the virus suspension used, despite the large numbers of particles, but a similarly prepared virus suspension has been shown to induce a wart on a human hand 10 weeks after inoculation (Cubie, 1972) and the preparation used here may be expected to be equally infective. It therefore seems likely that human skin grafts on 'nude' mice are not susceptible to infection by human papilloma virus, at least over the short incubation period available and under the conditions described above. Pass, Niimura & Kreider (1973) have likewise shown that human skin grafts on mice treated with antithymocytic serum failed to develop papillomas within a 14-week period.

While it had been hoped that direct contact of a high concentration of virus with well-traumatized skin might reduce and standardize the incubation period of wart virus, it seems unlikely in retrospect that 'nude' mice can be made to survive long enough to allow for a latent period variably estimated at between 6 weeks and 20 months (summarized in Rowson & Mahy, 1967). Nevertheless, the survival of the 'nude' mice in this study was disappointing, despite care to prevent contact with pathogens. Although Custer *et al.* (1973) and Croy & Osoba (1973) reported similar survivals in the range of 70–104 days in filter racks, Rygaard & Friis (1974) found average survivals of 202 days for their SPF-reared stock mice. The mice used here were all experimental animals and Reed & Manning (1973) certainly felt that the trauma of grafting and subsequent restriction with dressings noticeably shortened the life expectancy of their 'nude' mice. It would appear essential in this sort of study that even more care and attention be given to the rearing of 'nude' mice though this requirement may stretch the facilities of some laboratories too far. However, their unique defects could still make 'nude' mice a valuable tool in certain areas of virological and oncological research.

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REFERENCES

Abramson, C. (1975) Personal communication.

Billingham, R.E. & Medawar, P.B. (1951) The technique of free skin grafting in mammals. *Journal of experimental Biology*, **28**, 385.

Butel, J.S. (1972) Studies with human papilloma virus modelled after known Papovavirus systems. *Journal of the National Cancer Institute*, **48**, 285.

Croy, B.A. & Osoba, D. (1973) Nude mice—a model system for studying the cellular basis of the humoral immune response. *Cellular Immunology*, **9**, 306.

Cubie, H.A. (1972) Laboratory investigations in the study of human papilloma virus. *M.Sc. Thesis*. University of Edinburgh.

Cubie, H.A. (1974) Experiments with human papilloma virus in cell culture. *British Journal of Dermatology*, **91**, 569.

CUSTER, R.P., OUTZEN, H.C., EATON, G.J. & PREHN, R.T. (1973) Does the absence of immunological surveillance affect the tumour incidence in 'nude' mice? First recorded spontaneous lymphona in a 'nude' mouse. *Journal of the National Cancer Institute*, **51**, 707.

DE SOUSA, M.A.B., PARROTT, D.M.V. & PANTELOURIS, E.M. (1969) The lymphoid tissues in mice with congenital aplasia of the thymus. *Clinical and experimental Immunology*, **4**, 637.

FLANAGHAN, S.P. (1966) 'Nude', a new hairless gene with pleiotropic effects in the mouse. *Genetical Research*, **8**, 295.

GIOVANELLA, B.C. & STEHLIN, J.S. (1973) Heterotransplantation of human malignant tumours in 'nude' thymusless mice. 1. Breeding and Maintenance of 'nude' mice. *Journal of the National Cancer Institute*, **51**, 615.

KARNOWSKY, M.J. (1967) A formaldehyde–glutaraldehyde fixative of high osmolality for use in electron microscopy. *Journal of Cell Biology*, **27**, Abs. 270.

KREIDER, J.W. & BREEDIS, C. (1969) The susceptibility of foetal rat skin in different immunologic environments to neoplastic induction with Shope Papilloma virus. *Cancer Research*, **29**, 989.

MANNING, D.D., REED, N.D. & SHAFFER, C. (1973) Maintenance of skin xenografts of widely divergent phylogenetic origin on congenitally athymic (nude) mice. *Journal of experimental Medicine*, **138**, 488.

PANTELOURIS, E.M. (1968) Absence of thymus in a mouse mutant. *Nature*, **217**, 370.

PANTELOURIS, E.M. (1971) Observations on the immunobiology of 'nude' mice. *Immunology*, **20**, 247.

PASS, F., NIIMURA, M. & KREIDER, J.W. (1973) Prolonged survival of human skin xenografts on antithymocyte serum-treated mice: failure to produce verucae by inoculation with extracts of human warts. *Journal of investigative Dermatology*, **61**, 371.

REED, N.D. & MANNING, D.D. (1973) Long-term maintenance of normal skin on congenitally athymic (nude) mice. *Proceedings of the Society for experimental Biology and Medicine*, **143**, 350.

ROWSON, K.E.K. & MAHY, B.W.J. (1967) Human papova (wart) virus. *Bacteriological Reviews*, **31**, 110.

RYGAARD, J. (1969) Immunobiology of the mouse mutant 'nude'. *Acta pathologica et microbiologica scandinavica*, **77**, 761.

RYGAARD, J. (1974) Skin grafts in 'nude' mice. 3. Fate of grafts from man and donors of other taxonomic classes. *Acta pathologica et microbiologica scandinavica, A*, **82**, 105.

RYGAARD, J. & FRIIS, C.W. (1974) The husbandry of mice with congenital absence of the thymus (*nude* mice). *Zeitschrift für Versuchstierkunde*, Bd **16**, S1–10.

STONE, P.A. & LAWRENCE, J.G. (1973) Healing of tangentially excised and grafted burns in man. *British Journal of Plastic Surgery*, **26**, 20.

SUTHERLAND, A.B. (1975) Personal communication.

WORTIS, H.H., NEALSEN, S. & OWEN, J.J. (1971) Abnormal development of the thymus in 'nude' mice. *Journal of experimental Medicine*, **134**, 681.

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Pharmacology and Treatment

An assessment of methods of treating viral warts by comparative treatment trials based on a standard design

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SUMMARY

A series of eleven comparative wart treatment trials undertaken between 1969 and 1975 and involving 1802 patients is described. A method of coding provided groups of patients matched for age, type, number and duration of warts, within which treatments could be randomized. The variation in response to treatment was shown to be influenced significantly by these factors and the level of cure to depend on the proportions of the various response groups within the population under consideration. These proportions were found to vary geographically and at different periods. In all the trials the results were assessed at 12 weeks.

In the treatment of hand warts, the percentage cure of patients treated with liquid nitrogen fell significantly from 75 to 40% when the interval between freezings was increased from 3 to 4 weeks. The average number of freezings required for a cure was 3·1 amongst all patients cured by 6 or less freezings at intervals of 2 or 3 weeks.

In a two-centre trial there was no significant difference between the percentage cure of patients with hand warts treated with liquid nitrogen (69%) and of those applying a paint containing salicylic and lactic acids (SAL) (67%). Patients receiving both treatments concurrently did better (78%) but the difference was not found to be statistically significant.

In the treatment of simple plantar warts the percentage cure for the SAL paint (84%) was found to compare favourably with that for a podophyllin treatment (81%). Only one of the patients cured by the paint in that trial was found to have had a recurrence after 6 months. The paint was found to be satisfactory for use under general practice conditions. Additions to the formula did not alter its effectiveness.

In the treatment of mosaic plantar warts the overall percentage cure for the SAL paint in a series of comparative trials (1969–75) was 45%. In these trials it was compared directly with one or more other preparations. No differences were found between its efficacy and that of 10% buffered gluteraldehyde (47%), 40% benzalkonium chloride dibromide (Callusolve 40) (30%) and 5% 5-fluorouracil in dimethyl sulphoxide (53%). Only 25% of thirty-six patients treated with 5% idoxuridine in dimethyl sulphoxide were cured.

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Throughout the trials approximately 30% of patients with hand warts, 20% of those with simple plantar warts and 50% of those with mosaic plantar warts were found to be resistant to treatment.

The adoption of treatment with SAL paint for hand warts and simple plantar warts by the general practitioners in the Edinburgh area has proved satisfactory. Only resistant cases are now referred to hospital and these can be treated within a few weeks instead of 4–5 months as was the case in 1969.

During the last 30 years the demands made on dermatological departments in Western Europe for the treatment of warts has risen from 4 to 25% of all cases referred (Rook, 1972). Hospital treatment for a self-limiting condition is expensive and time consuming not only to the Health Service but also to the patient, who may have to lose work or schooling and make long costly journeys.

The local situation at Edinburgh Royal Infirmary in 1969 was that, although 5000 wart treatments were given each year, patients were having to wait up to 5 months for a first appointment. An effective alternative to hospital referral which could be confidently recommended to general practitioners was required. Claims for the success of wart cures must be viewed with caution unless comparisons are made directly on groups of patients matched for age and for number, type and duration of warts, as all these factors influence the response to treatment and spontaneous resolution. A treatment can only be considered effective if the level of cure is higher than that by spontaneous resolution within the same period of time.

A standard design for comparative wart treatment trials was devised to allow for variations in response. A series of eleven trials involving 1802 patients have been completed between 1969 and 1975, to assess the effectiveness of the treatments given in our wart clinics and of preparations suitable for self-application under the supervision of the general practitioner. One of these trials was carried out in two centres in collaboration with the Department of Dermatology, Royal Infirmary, Dundee.

The choice of methods investigated has been influenced by the conviction that treatment of warts should not endanger the patient or leave scarring, and cause as little discomfort and inconvenience as possible.

For simplicity the trials have been grouped according to the site of warts under treatment rather than chronologically. Those with results considered of practical importance in the management of warts are described in detail. Any relevant information has been abstracted from the rest.

THE BASIC DESIGN OF TRIALS

(1) New patients attending the wart clinics were included in a trial providing they only had the category of warts under investigation.

(2) To obtain comparable response groups of patients each was given a 3-lettered code. The first letter represented the age of the patient—under 5 years (A); 5–18 years (B); over 18 years (C). The second letter indicated whether warts were single (S) or multiple (M), and the third the type of wart, simple or common (S) or mosaic (M). If the warts had been present for more than 6 months the figure 6 was added. After the first trial it was found that there were too few children under 5 to warrant a separate group, so all patients under 18 were coded B. All patients with the same code, e.g. BSS, comprised a response group.

(3) Treatments were allocated from a separate random table for each response group. Treatments were allocated blind when possible, the key being held in the hospital pharmacy until the end of the trial.

(4) Documentation was made on a standard sheet for inclusion in the hospital case notes, a carbon copy being filed separately.

(5) When patients were treating their own warts, the method of treatment was demonstrated and written instructions given so that it was thoroughly understood.

(6) Patients attended at set intervals for treatment or review.

(7) The duration of a trial for each patient was 12 weeks. A trial was concluded when each treatment had been allocated to sufficient patients to anticipate a valid result.

(8) At the end of 12 weeks each patient was assessed 'cured', 'failed', 'rejected' or 'unknown'. The criterion of cure was complete restoration of the skin ridge lines in the area of the wart on examination with a magnifying glass. Patients were rejected if they failed to attend regularly or were withdrawn on account of complications or persistent pain. On completion of a trial patients who had failed to attend were circularized to establish whether the warts were cured or remained.

The results of a trial were entered on a standard table designed to facilitate the study of the fate of all the patients in the trial or in a particular group or groups so that any inequalities would be immediately seen and could be investigated for contributory factors. It also provided a cross-check that the response groups were equally distributed between treatments. The percentage cure at 12 weeks was calculated and usually the percentage of cured patients who were already cured at 6 weeks was also estimated to indicate speed of cure. The results were interpreted by the statistician who also prepared the random tables.

The salicylic acid paint (SAL) used in the trials contained salicylic acid 1 part: lactic acid 1 part: flexible collodion 4 parts and was made up in the hospital pharmacy.

HAND WART TREATMENT TRIALS

LIQUID NITROGEN TREATMENT TRIAL (1971–72)

Aim. (1) To establish the effectiveness of freezing with liquid nitrogen in the cure of hand warts.

(2) To investigate the influence of frequency of treatment on the speed of cure.

Method. (1) The basic trial design was modified only in that there were insufficient patients with warts present for less than 6 months to warrant separate duration coding, because of the long time they had been on the waiting list.

TABLE 1. Results at 12 weeks for patients with hand warts treated by freezing with liquid nitrogen at varying intervals

Patients	2-weekly	3-weekly	4-weekly
Total treated	34	31	35
Rejected or untraced	11	7	10
Failed	5	6	15
Cured	18	18	10
% cured	78·2	75·0	40·0

(2) From the random tables, patients were allocated treatment at intervals of 2, 3 or 4 weeks respectively.

(3) To standardize freezing technique as far as possible, all treatments were carried out by one of us (MHB). Liquid nitrogen was applied with a cotton wool bud just smaller than the wart, using slight vertical pressure, until a frozen halo appeared around its base (5–30 s according to the thickness of the wart). All warts present were treated.

Results. One hundred patients were admitted to the trial. Sixteen were rejected because of irregular attendance. Twelve defaulters remained untraced. The results for the remaining seventy-two patients are shown in Table 1. The percentages of patients cured are very similar, for patients treated at intervals of 2 weeks (78%) and for those treated at 3 weeks (75%), but there was a statistically significant ($P = 0{\cdot}014$) drop to 40% for patients treated at intervals of 4 weeks.

Patients in the three groups had received different numbers of freezings, 6, 4 and 3 respectively. The question therefore arose as to whether these differences were attributable to the frequency of treatment or the number of freezings received. As treatment of uncured patients had been continued after their trial period it was possible to plot the percentages of patients cured against the number of freezings for the three regimes (Fig. 1). The response curves for 2- and 3-weekly freezings are similar and lie above the 4-weekly response curve. Although the response curves do not differ to a statistically significant extent they suggest that the differences are due to the interval between freezings rather than the number of freezings received. 83% of the forty-seven patients treated at intervals of 2 or 3 weeks were cured by 6 freezings; the average number of treatments required was 3·1.

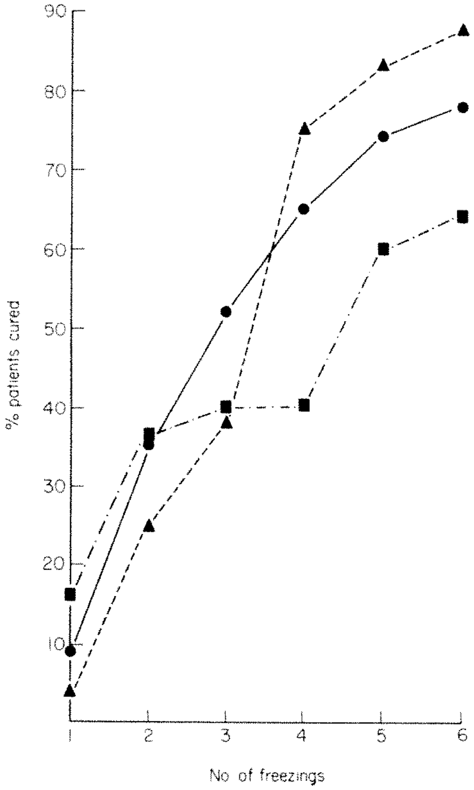

FIGURE 1. Percentage of patients cured by 1–6 freezings with liquid nitrogen related to the interval between freezings. ● 2-weekly; ▲ 3-weekly; ■ 4-weekly freezings.

Conclusions. (1) 70–80% of patients with hand warts can be cured by treatment with liquid nitrogen within 12 weeks providing the interval between treatments is not more than 3 weeks.

(2) Patients are unlikely to be cured by less than three treatments.

EDINBURGH/DUNDEE HAND WART TREATMENT TRIAL (1973–74)

Aim. To compare the effectiveness in curing hand warts of freezing with liquid nitrogen, the application of SAL paint by the patient, and a combination of both methods.

Material and method. (1) The trial was carried out over the same period in Edinburgh and Dundee.

(2) The same protocol was followed in both centres following the basic trial design. As in the previous trial, duration coding was omitted.

(3) From the random tables patients were allocated treatment by the application of SAL paint, freezing with liquid nitrogen alone or freezing combined with the use of SAL paint in the interval between freezings.

(4) Patients allocated SAL paint were shown how to use it and given typed instructions as follows: (i) Wash hands thoroughly with soap and water; (ii) Rub surface of wart gently with manicure emery board; (iii) Apply paint carefully to the wart with a sharpened matchstick or orange stick; (iv) Allow to dry; (v) Keep bottle tightly closed; (vi) Repeat each night. *Note*: Do not apply the paint to any warts treated with liquid nitrogen at hospital until the inflammation has settled down.

(5) All patients were seen at intervals of 3 weeks when warts were frozen with liquid nitrogen or progress reviewed. Freezing treatment was carried out by medical staff of the two dermatological departments and the technique was standardized as far as possible.

Results. 389 patients were admitted to the trial (252 in Edinburgh and 137 in Dundee). 134 patients were rejected principally on account of irregular attendance, only 31 defaulters remained untraced. The results for the remaining 294 patients are shown in Table 2.

TABLE 2. Results at 12 weeks for 294 patients with hand warts treated with SAL paint, liquid nitrogen and a combination of the two

Patients	SAL paint only			Liquid nitrogen only			Combined treatment		
	E	D	Total	E	D	Total	E	D	Total
Total assessed	59	36	95	60	39	99	64	36	100
Failed	25	6	31	26	5	31	18	4	22
Cured	34	30	64	34	34	68	46	32	78
% cured	57·6	83·3	67·3	56·6	87·1	68·6	71·8	88·8	78·0

E = Edinburgh; D = Dundee.

Whilst the level of cure in the two centres was different, there was little difference between the percentages cured by SAL paint alone and by liquid nitrogen alone, in Edinburgh 58:57%, in Dundee 83:87%, or as a whole 67:69% whereas that for the combined treatment was higher, 72, 89 and 78% respectively. However, further analysis of the results by the method described by Mantel (1966) showed that this apparent superiority of the combined treatment was not statistically significant ($P = 0{\cdot}1$).

The tabulated results of the trial were examined for an explanation of the different levels of cure in the two centres noted in all three treatment groups.

At each centre the method of allocating treatments to patients ensures that the proportionate division of patients into their response groups is the same for each treatment. These proportions do, however, differ considerably between centres, as shown in Table 3. In Dundee there were appreciably more responsive single simple and fewer resistant mosaic wart patients.

TABLE 3. Variation in percentage of hand wart types between Edinburgh and Dundee

Wart type	% of patients	
	Edinburgh	Dundee
Single simple	9·5	30·6
Multiple simple	48·8	40·1
Mosaic	41·6	29·1

The pattern of duration of warts in the two centres was studied from the record sheets and is shown in Table 4. Again the greater number of warts of short duration and smaller number of persistent warts in Dundee were in favour of a higher percentage cure in that centre.

Conclusion. (1) The use of the SAL paint by patients at home cured as many patients within 12 weeks as did treatment with liquid nitrogen.

(2) The application of SAL paint between treatments with liquid nitrogen possibly increases their effectiveness.

(3) The level of cure achieved by wart treatments is significantly influenced by the response pattern of the patients upon whom it is used.

TABLE 4. Comparison of wart duration pattern in patients presenting for treatment in Edinburgh and Dundee

Duration	% of patients	
	Edinburgh	Dundee
Less than 1 year	23·3	46·0
1–2 years	32·6	38·0
2 years or more	44·0	15·8

ABSTRACT: HAND WART PAINT TREATMENT TRIAL (1970–71)

In this trial the effectiveness of three wart paints was compared on 272 patients. Analyses of results showed no statistically significant difference between the percentage of patients cured at 12 weeks by Acid Salicylic Collodion N.F. (54%), Flexible Collodion B.P. (52%) and a non-irritant preparation containing benzalkonium and cetylpyridium bromides 'Verucosol' (59%). The factor influencing the speed of cure was the type of wart. This was obtained by combining the cures in all groups and estimating the percentage of patients cured at 12 weeks who were already cured at 6 weeks, which was 62% for single simple warts, 39% for multiple simple warts and 21% for mosaic types.

PLANTAR WART TREATMENT TRIALS

ABSTRACT: THE 1969 PLANTAR WART TREATMENT TRIAL

This trial has been fully reported elsewhere (Bunney *et al.*, 1970). 382 patients were admitted to the trial. 296 of these had simple plantar warts. The percentage of these patients cured at 12 weeks by the use of SAL paint was 84% (89% for children). This compared favourably with that for the podophyllin-occlusion method in current use in our department (81%). It had a higher speed of cure and fewer side effects. It was significantly more effective than Collodion Flex B.P. (64%), or 'Callusolve', a preparation containing 25% benzalkonium chloride dibromide (67%).

The written instructions given to the patients for the application of all the paints are considered of importance and were also used in all subsequent plantar wart trials and were as follows: *Each night* (i) Remove the plaster; (ii) Rub away the surface of the wart with a manicure emery board or pumice stone; (iii) Soak the foot in hot water or your bath for at least 5 min (use your own towel and bath mat); (iv) Apply a drop of paint to the surface of the wart with a pointed matchstick. Allow it to dry in. If the wart is large, apply another drop; (v) When dry, cover the area with a piece of sticking plaster; (vi) Every 3 weeks consult your doctor to find out if the warts are cured; (vii) It may be longer than 3 weeks before your warts go away, but they will go if you keep on with the treatment regularly. The doctor will tell you when to stop; (viii) *If your foot becomes painful, stop treatment until you have seen a doctor.*

The results for mosaic plantar warts showed that the level of cure was so different from that for simple plantar warts that they were better investigated separately. There were insufficient patients with this type of wart to provide a valid result.

The conclusion was that the SAL paint applied nightly by the patient at home was a satisfactory method of treating simple plantar warts.

FOLLOW-UP STUDIES ON THE USE OF SAL PAINT

Subsequent to publication of this trial further investigations into the effectiveness of SAL paint were carried out. The recurrence rate, conditions of use and the effect of additives were studied.

Recurrence rate. Any assessment of cure must take into account the possibility of recurrence. It was considered that the standard of cure adopted in these trials was reliable and would not be followed by a high recurrence rate. To test this the sixty-four patients who were 'cured' by the use of the SAL paint were circularized 6 months after the conclusion of the trial. Forty-one replied: only one had suffered a recurrence.

Conditions of use. Owing to the enthusiasm of the researcher and consequent interest of patients the results obtained under trial conditions are frequently better than when the same preparation is used in the normal clinical situation. Following the trial it had been decided that new patients with simple plantar warts should be prescribed the SAL paint and referred back to their general practitioners for supervision. Fifty of these patients were traced about 6 months after starting treatment: forty-six (92%) reported that their warts had been cured; one had had a recurrence and in three the warts remained.

VERSAL PLANTAR WART TREATMENT TRIAL (1974–75)

Aim. To discover whether the addition of polyoxyethylene/lauryl/ether to the SAL formula Versal would, by increasing penetration, improve its effectiveness.

Material and method. The basic trial design was followed. Patients with simple plantar warts were allocated treatment with 'Versal' or SAL paint from the random tables. Instructions for application were the same as for the 1969 trial.

Results. 156 patients were admitted to the trial. Twelve patients were rejected because they failed to attend, or to carry out treatment regularly. Only one patient in each treatment group was rejected on account of persistent pain or failure to improve. Six defaulters could not be traced. The results for the remaining 138 are shown in Table 5. There was no significant evidence for difference between the efficacies of Versal (75%) and the SAL paint (77%).

TABLE 5. Results at 12 weeks for patients with simple plantar warts treated with Versal or SAL paint

Patients	Versal	SAL paint
Total assessed	67	71
Failed	17	16
Cured at 6 weeks	23	21
Cured at 12 weeks	50	55
% cured at 12 weeks	74·6	77·4

Conclusion. The addition of polyoxyethylene/lauryl/ether did not influence the effectiveness of the SAL formula.

Abstracts: verucosol and verrucid simple plantar wart trials

(1) 'Verucosol' (1970–71). The non-irritant preparation previously described was found to cure significantly less patients in 12 weeks (70%) than the SAL paint (91%).

(2) 'Verrucid' (1973–74). A preparation containing salicylic acid and laricinol resins showed no difference in effectiveness (76%) from the SAL paint (74%).

MOSAIC PLANTAR WART TREATMENT TRIALS

The overall response to treatment of mosaic warts in the 1969 plantar wart trial was significantly lower (58%) than for other types (78%). There were too few patients with mosaic warts to show differences between treatments. The search for an effective treatment for mosaic warts was continued in a series of comparative trials using the SAL paint as a control.

ABSTRACT: 5-FLUOROURACIL (5-FU) AND IDOXURIDINE MOSAIC PLANTAR WART TRIALS (1970–72)

Aims. To study the effectiveness in the treatment of mosaic plantar warts of:

(1) Topical 5-FU which had been reported by Goldman Blaney & Cohen (1962) as being successful in the treatment of warts.

(2) Topical idoxuridine which can prevent viral replication by creating weak or faulty links in viral DNA.

Material and methods. The basic trial design was modified to increase the number of patients in each treatment group. Patients failing the trial on one treatment were readmitted on one of the others allocated from specially prepared follow-on random tables. The preparations used were: 2% 5-FU in propylene glycol (equal in potency with 5% ointment); 5% 5-FU in dimethyl sulphoxide (DMSO); 5% idoxuridine in DMSO. SAL paint and 'Verucosol' were also included. The preparations were applied by the patients in the way previously described for SAL paint.

Results. The results relevant to 5-FU have already been reported (Bunney, 1973). There was no difference in efficacy between 5% 5-FU in DMSO (53%) and the SAL paint (50%). 5% idoxuridine in DMSO was used on thirty-six patients of whom only 25% were cured compared with 46% who were cured by 2% 5-FU in propylene glycol.

Conclusion. There was no evidence that any of the preparations of 5-FU or idoxuridine tested was more effective against mosaic plantar warts than the SAL paint.

GLUTERALDEHYDE MOSAIC PLANTAR WART TRIAL (1973–74)

Following encouraging reports of the successful use of buffered gluteraldehyde by Nelson (1972) in California, a clinical trial of the preparation was authorized.

Material and method. The unmodified basic trial design was used. A 10% buffered solution of gluteraldehyde was prepared by mixing equal parts of 20% aqueous gluteraldehyde with an aqueous solution containing 2·3% sodium bicarbonate with 0·2% Tween 80. As gluteraldehyde polymerizes in buffered solution within 3–4 weeks, it was necessary to mix the solution freshly on each occasion the patients attended. The gluteraldehyde was compared directly with the SAL paint.

TABLE 6. Results at 12 weeks for patients with mosaic plantar warts treated with 10% gluteraldehyde or SAL paint

Patients	Gluteraldehyde	SAL paint
Total assessed	38	43
Failed	20	24
Cured at 6 weeks	4	7
Cured at 12 weeks	18	19
% cured at 12 weeks	47·3	44·2

Results. Ninety-four patients were admitted to the trial. Twelve were rejected and one remained untraced. The results of the remaining eighty-one patients are shown in Table 6. There was no significant evidence of a difference between the efficacies of the gluteraldehyde (47% cure) and SAL paint (44% cure).

Conclusion. The gluteraldehyde is no more effective than SAL paint and the problems of dispensing are considerable.

CALLUSOLVE 40 MOSAIC PLANTAR WART TRIAL (1974–75)

In the 1969 plantar wart trial Callusolve appeared to be more effective in the cure of mosaic warts than the other preparations under test but it was emphasized that the numbers involved were too small to be significant. A further trial was therefore undertaken to investigate the effectiveness of Callusolve 40.

Material and method. Callusolve 40 in which the active ingredient was increased to 40% was directly compared with SAL paint using the unmodified basic trial design.

Results. 110 patients were admitted to the trial. Thirteen were rejected and four remained untraced at the end of the trial. The results at 12 weeks are shown in Table 7. A percentage cure at 12 weeks of 30% for Callusolve 40 and 40% for SAL paint was obtained in this group of patients. The difference is not statistically significant.

TABLE 7. Results at 12 weeks for patients with mosaic plantar warts treated with Callusolve 40 or SAL paint

Patients	Callusolve 40	SAL paint
Total assessed	50	43
Failed	35	26
Cured at 6 weeks	5	4
Cured at 12 weeks	15	17
% cured at 12 weeks	30·0	39·5

Conclusion. Callusolve 40 is not more effective in the treatment of mosaic plantar warts than the SAL paint.

THE LEVEL OF CURE FOR SAL PAINT IN THE TREATMENT OF MOSAIC WARTS

Between 1969 and 1975 the percentage of patients cured by the use of SAL paint in the mosaic wart trials fell from 50 to 40%. This could be explained by the change in incidence of the response groups recorded in Table 8. Whilst there was no significant variation in the proportion of children (B Groups) the number of patients with mosaic warts of more than 6-months duration (M6 Groups) increased appreciably from 50% in 1969 to 86% in 1974. This provides further evidence of the significant effect of the patients' response group on the success of the treatment.

TABLE 8. Change in referral pattern of patients with mosaic plantar warts between 1969 and 1975

Period of trial	No. of patients in trial	% Children	% Response group M6
1969–70	52	61·5	50·0
1970–71	80	52·5	76·3
1971–72	44	29·5	84·1
1973–74	94	40·5	82·0
1974–75	110	52·0	86·4

DISCUSSION

Basic design. Since these trials were started there has been growing evidence that the cure of wart infection is a complicated process depending on the immunological competence of the patient, the amount and availability of the virus and possibly the life-span of the virus itself. Cell-mediated immunity and circulating antibodies develop to both the virus and the wart cell. The role of each in the regression of warts has yet to be resolved. Recent studies by Pyrhönen & Johansson (1975) and Shirodaria & Matthews (1975) and others have demonstrated that the amount of virus present and the development of antibodies differ with the number, type, duration and site of warts. This provides some explanation for the wide variations in the response to treatment anticipated and confirmed in our trials. The results of the Edinburgh/Dundee trial showed clearly that even when quite large numbers of patients are involved, the level of cure depends on the proportion of the various response groups within the 'populations' under study at any one time. The mosaic wart trials showed that these variations could also occur with time. They emphasize that valid conclusions cannot be drawn about relative effectiveness of treatments unless they are compared directly on matched groups of patients. The method of coding of patients used in these trials proved successful in providing such matched groups between which treatments could be randomized.

The recurrence rate of warts is known to be high; Rulison (1942) estimated it to be about 5% for common warts, occurring within 3–4 months. Many hold the view that an assessment of the recurrence rate should be included in all treatment trials. Unfortunately, follow-up in large trials is expensive, time consuming and often unproductive. The most common cause of recurrence is probably incomplete cure in the first place. The restoration of the normal skin texture established by examination with a magnifying glass is a positive criterion of cure, not previously mentioned in treatment trials, although Hursh (1968) considered a break in the continuity of the epidermal ridge pattern an indication for the continuation of treatment with liquid nitrogen. Whilst there will be human error, evidence that this is a reliable standard of cure was given by the single recurrence reported in the follow up of the 1969 plantar wart trial. Further, the case notes of all patients referred back to the department with recurrent warts of any type between September 1972 and December 1974 were studied. There were thirty-nine such patients, a small number considering 1000 new wart patients are treated each year. Only eight of these patients were assessed cured at their last previous visit compared with thirty-one who had assessed their own cure. This inaccurate self-assessment of cure questions the validity of including such assessment by defaulters in trial results. The tendency of patients to stop attending if they believe their warts to 'have gone' leads to a high defaulter rate and prolongation of trials unless such results are used. If cure has not in fact occurred warts will reappear within 2 or 3 months so that by contacting defaulters after this interval an acceptably accurate assessment was considered likely. The proportion of defaulters was balanced within the treatment groups. There is no reason to suppose that errors in assessment would not also be equally distributed.

Hand wart trials. Cryotherapy of warts has been practised successfully for many years. Most of the techniques used aim at the production of a blister beneath the wart. Whilst this can be tolerated if only one or two warts are present, it is incapacitating in the case of multiple warts. The technique used in these trials reduces blistering to a minimum. All but the hard core of resistant patients were cured by three or four treatments which is in agreement with the study of Barr & Coles (1969) who also are of the opinion that blister production is unnecessary. It may indeed be undesirable to cure warts by a single treatment in view of the finding by Morison (1974) that a change in immune status only occurred in patients cured by several treatments. Bellows (1967) postulated that whilst viruses are preserved by an initial rapid freezing they are destroyed by cyclic freezing and thawing. It is difficult to provide an explanation for the significant drop in cure rate observed when the interval between freezings is extended from 3 to 4 weeks. This could be related to wart cell turnover rate or to the rate of wart virus replication about both of which little is known. The number of freezings received may also be contributory. This is an important observation and indicates that, for maximum efficiency, warts should be frozen at intervals of not more than 3 weeks.

Freezing, especially in the case of multiple warts, can be most painful. It was therefore gratifying if surprising to find that within the period of the 12 weeks usually required to cure warts by freezing just as many patients were cured by the self-application of the SAL paint. This applied even to patients with widespread periungual warts. The combination of both methods of treatment cleared warts rather more quickly but not to a significant level. The importance of proper instruction and supervision of the patient using a wart paint cannot be too highly stressed. In the course of the trials it was found repeatedly that a paint, previously used inadequately, was successful when properly applied. These trials provide sufficient evidence to recommend that patients with hand warts should first be treated with a wart paint such as SAL for a period of 3 months. Adoption of this practice by the general practitioners in the Edinburgh area has resulted in the reduction of the time patients have to wait to attend our wart clinics from 4 to 5 months in 1969 to as many weeks in 1975.

Simple plantar wart trials. Subsequent trials of this series confirmed the findings of the 1969 trial that the use of the SAL paint was successful in the treatment of simple plantar warts in at least 80% of cases. Pain is rapidly relieved and few direct side effects were observed. When soreness occurred this was usually due to excessive application of paint, and disappeared when the paint was properly applied. The discomfort could in no way be compared with that experienced when podophyllin preparations were used. Some cases of sensitivity to adhesive plaster were revealed, necessitating the substitution of 'Micropore' tape. The success of this method of treatment has resulted in a dramatic fall in the number of cases of simple plantar warts referred to our wart clinics reflected in the reduction of the waiting time for a new appointment from 3 to 4 months in 1969 to an immediate appointment in 1975.

Mosaic plantar wart trials. With the exception of the mosaic section of the 1969 trial which was too small to provide significant results no level of cure higher than 53% within the 12 weeks was recorded. The resistance of mosaic warts to treatment must be associated with the small quantity of virus present, and available for release by treatment, and consequential poor stimulation of the immune systems. Morison (1975) found that even 40% idoxuridine in DMSO was unsuccessful but had success with 20% idoxuridine in a cream base used under occlusion. He suggests that DMSO carries the drug through the epidermis too rapidly to allow it to attack the virus. The use of idoxuridine in a cream base is worth further investigation although expense would exclude its use except in a few really resistant and widespread cases with known immunological deficiency. Hursthouse (1975) has used 5% 5-fluorouracil ointment in a bilateral trial and obtained only a 60% cure rate in mixed wart population and agrees with us that 5-fluorouracil has no advantage over other methods and should be reserved for the exceptional case. 10% gluteraldehyde solution showed no advantage over SAL paint and there were considerable dispensing problems. Formalin treatment was not included formally in this series of trials because of the mechanical difficulties of keeping the solution away from less keratinized areas of the skin of the foot and the danger of sensitizing the patient to a chemical now widely used in the processing of clothing materials. Barr & Coles (1966) recorded a 50% cure rate for mosaic warts in their series and emphasized the importance of the choice of painless treatments for this type of wart. Vigorous radical treatments are no more rewarding. Our results certainly reinforce this opinion.

Wart paints. Present information on the mechanisms responsible for the cure of warts suggests that keratolytic agents such as salicylic acid act both by the mechanical removal of infected cells and wart virus and also, by provoking a mild inflammatory reaction, so rendering the virus more readily accessible to the forces responsible for the development of immune status. Lactic acid has been used for many years in the treatment of warts (Stelwagon & Gaskill, 1923) and is still included in the formula of wart paints appearing in some pharmacopoeiae. The SAL paint used in the trials was already in use in our department and elsewhere. The wart paints tested in these trials which did not contain salicylic acid were significantly less effective.

Resistant warts. In these trials approximately 70% of common, 80% simple plantar warts and 50% of mosaic plantar warts were cured within 12 weeks by simple methods. This leaves a hard core of patients with resistant warts. Some of these patients have reduced or absent immunological competence or are on immunosuppressive drugs and have widespread warts which militates against surgical interference. For these patients individual and more sophisticated techniques must be explored.

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REFERENCES

Barr, A. & Coles, R.B. (1966) Plantar warts: a statistical survey. *Transactions of St John's Hospital Dermatological Society*, **52**, 226.

Barr, A. & Coles, R.B. (1969) Warts on the hands: a statistical survey. *Transactions of St John's Hospital Dermatological Society*, **55**, 69.

Bellows, J.G. (1967) Cryotherapy destroys virus in cornea. *Medical World News*, **8**, 34.

Bunney, M.H., Hunter, J.A.A., Ogilvie, M.M. & Williams, D.A. (1971) Treatment of plantar warts in the home. A critical appraisal of a new preparation. *The Practitioner*, **207**, 197.

Bunney, M.H. (1973) The treatment of plantar warts with 5-Fluorouracil. *British Journal of Dermatology*, **89**, 96.

Hursh, L.M. (1968) Wart cryotherapy. *Journal of Cryosurgery*, **1**, 280.

Hursthouse, M.W. (1975) A controlled trial on the use of topical 5-fluorouracil on viral warts. *British Journal of Dermatology*, **92**, 93.

Goldman, L., Blaney, D.J. & Cohen, W. (1962) Studies of warts. *Proceedings of XII International Congress of Dermatologists*, **2**, 1522.

Mantel, N. (1966) Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemotherapy Reports*, **50**, 163.

Morison, W.L. (1974) *In vitro* assay of cell-mediated immunity to human wart antigen. *British Journal of Dermatology*, **90**, 531.

Morison, W.L. (1975) Anti-viral treatment of warts. *British Journal of Dermatology*, **92**, 97.

Nelson, L.M. (1972) Personal communication.

Pyrhönen, S. & Johansson, E. (1975) Regression of warts: an immunological study. *Lancet*, **i**, 592.

Shirodaria, P.V. & Matthews R.S. (1975) An immunofluorescence study of warts. *Journal of Clinical and Experimental Immunology*, **21**, 29.

Stelwagon, H.W. & Gaskill, H.K. (1923) *Diseases of the Skin*. 9th edn, p. 580. W. B. Saunders Philadelphia.

Rook, A., Wilkinson, D.S. & Ebling, F.J.G (1972) *Textbook of Dermatology*, 2nd edn, Vol. 1, p. 550. Blackwell Scientific Publications, Oxford.

Rulison R.H. (1942) Warts. *Archives of Dermatology and Syphilology*, **46**, 66.

British Journal of Dermatology (1976) **94**, 681.

Pemphigus controlled by dapsone

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SUMMARY

Three uncomplicated cases of pemphigus were clinically controlled by dapsone. Improvement was associated with decreasing titres of circulating intercellular antibodies. Interestingly, the sera from one case of pemphigus foliaceus contained intercellular antibodies found in the Malpighian and basal cell layers using the fluorescent technique and in the granular layer using the peroxidase technique. These findings suggest that the intercellular antibodies in pemphigus vulgaris and pemphigus foliaceus are similar but bind at different anatomical sites.

Many cases have been reported of mixed bullous diseases, clinically diagnosed as dermatitis herpetiformis associated with pemphigus and responding to sulphonamides (Floden & Gentele, 1955; Winkelmann & Roth, 1960; Doepfmer, 1961; DeMento & Grover, 1973; Seah *et al.*, 1973; Barranco, 1974a). The latter diagnosis was confirmed by immunofluorescent techniques in the last three reports, casting doubt on whether the former diagnosis in such cases might have been clinically and histologically correct, as Emmerson & Wilson Jones (1968) suggested, even though all the cases responded to sulphonamides.

In this communication three cases are reported in which a definite diagnosis of pemphigus was made. These cases were controlled by dapsone and there were decreasing titres of intercellular antibodies.

METHODS

The clinical diagnosis of pemphigus was confirmed in all cases by Tzanck's smear, histology, and direct and indirect immunofluorescent tests.

Direct immunofluorescent studies. Skin biopsies taken from vesiculobullous lesions were immediately sectioned at 4 μm in a cryostat at -20°C and incubated with fluorescein conjugated antihuman IgG, IgA, IgM, and C'_3 (Hyland Laboratories) at 1:4 dilution (the fluorescein protein ratio of IgG was 7·7 μg/mg, IgA 6·0 μg/mg, IgM 7·4 μg/mg; C'_3 8·3 μg/mg).

Indirect studies. Sections from normal and hyperkeratotic human skin as substrate were incubated with pemphigus sera and stained with fluorescein conjugated antihuman IgG, and also peroxidase antihuman IgG. The staining procedures for immunofluorescent tests were performed according to the techniques described by Beutner, Chorzelski & Jordon (1970) and for peroxidase tests by Fellner *et al.* (1973).

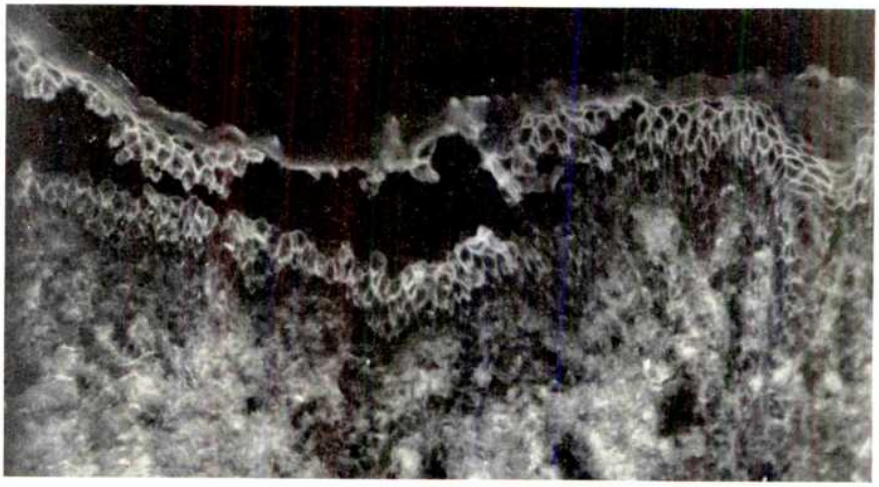

FIGURE 1. Positive direct immunofluorescent test for intercellular antibody. Case 1 (Magnification × 200).

CASE REPORTS

Case 1

A 30-year-old Thai male was admitted to the Institute of Dermatology, Bangkok, on 25 November 1974 with a history of a vesicular eruption on his face, trunk, and extremities for 3 years. Previous treatment from a local physician gave him no relief. The lesions were crusted and denuded, with confluent vesicles on either normal or erythematous skin on his face, chest, back and extremities.

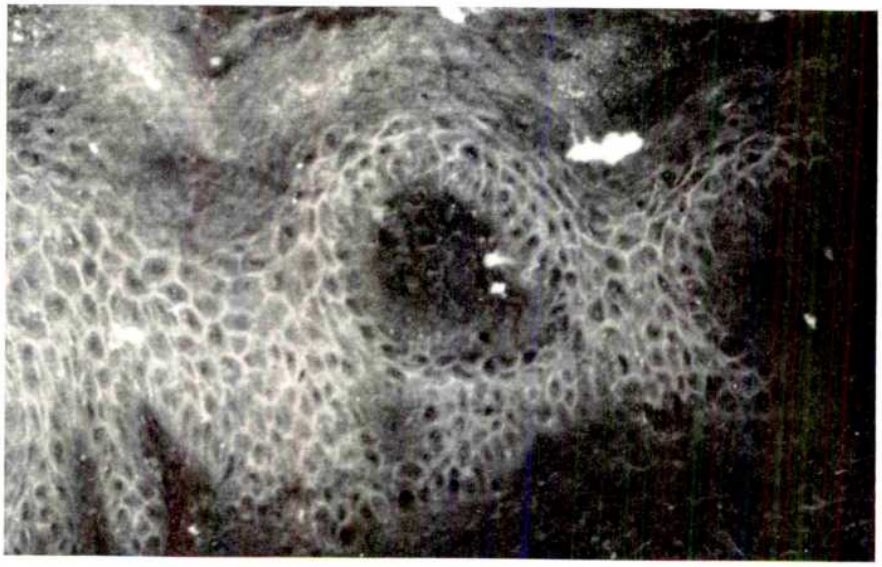

FIGURE 2. Indirect immunofluorescent test: presence of intercellular antibody in the Malpighian and basal cell layers using hyperkeratotic human skin as substrate (Magnification × 200).

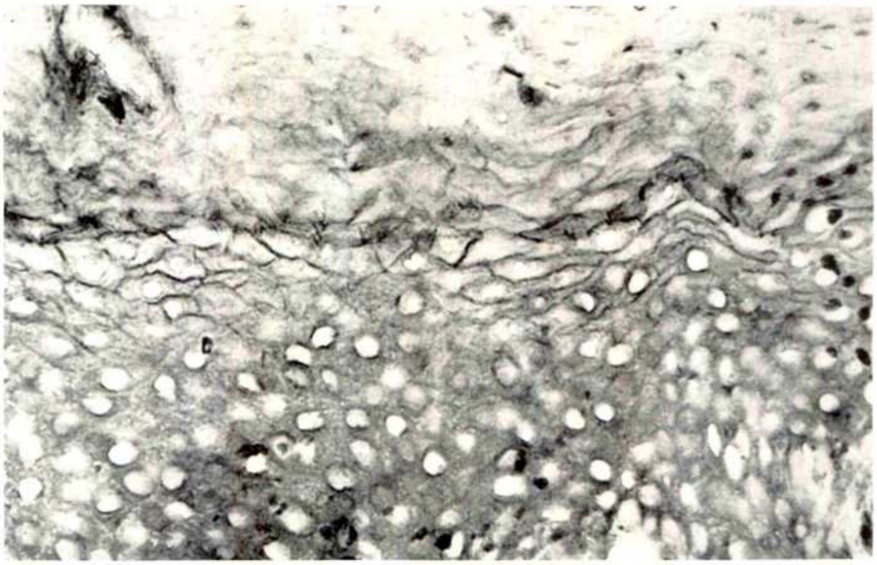

FIGURE 3. Indirect peroxidase staining with serum from Case 1 using hyperkeratotic human skin as substrate. Intercellular localization of IgG in the granular layer is demonstrated (Magnification × 400).

The diagnosis of pemphigus foliaceus was made clinically and confirmed by the presence of acantholytic cells in the Tzanck's smear. Direct and indirect immunofluorescent tests on the day of admission revealed intercellular IgG antibodies in the Malpighian and basal cell layers with a titre of 1:160 (Figs 1 and 2). The indirect peroxidase test showed intercellular staining in the granular layer, using hyperkeratotic human skin as substrate (Fig. 3). Histological examination showed a small separation in the granular layer with acantholysis. His blood group is B. Dapsone 100 mg/d was initiated on 30 November 1974. He promptly responded to treatment within 2 weeks, and no skin lesions have been observed since. He was discharged on 16 December 1974 with the same titres. Afterwards, the circulating titres slowly decreased and the dose of dapsone was gradually tapered as an out-patient (Table 1).

Case 2

A 40-year-old Thai male, a case of pemphigus erythematosus, had been kept under control by prednisone and cyclophosphamide since 28 November 1974.

On 16 January he suddenly developed signs of hepatotoxicity, and cyclophosphamide was discontinued. Prednisone 15 mg/d was unable to control the disease. The titre of circulating antibodies increased to 1:80 (31 January 1975). At that time dapsone 100 mg/d was added. Over a few weeks he responded to this combined treatment. At present, he has no lesions and the last titre was 1:40 (Table 1). His blood group is B.

Case 3

A 28-year-old Thai male with pemphigus vulgaris for 1 year was admitted on 28 November 1974. Prednisone 60 mg/d had been given for only 1 week, when he suddenly developed acute appendicitis and underwent operation. The incision wound was not healing, so prednisone was rapidly tapered to 10 mg/d and dapsone 100 mg/d was added. He responded very well, the wound healed, and he has

TABLE 1. Case summaries

Date	Dose of dapsone/day (mg)	Lesions	Titres of intercellular antibodies
Case 1			
25/11/74	100	Active	1:160
13/12/74	100	No	1:160
10/1/75	100	No	1:160
31/1/75	100	No	1:80
7/2/75	100	No	1:40
7/3/75	100	No	1:40
4/4/75	80	No	1:20
2/5/75	40	No	1:10
28/5/75	20	No	1:10
Case 2			
28/11/74	Prednisone 60 mg & cyclophosphamide 50–100 mg	Active	1:160
13/12/74	Same	Active	1:80
16/1/75	Prednisone 15 mg & cyclophosphamide 50 mg every other day	No (Hepatotoxicity was noted)	1:20
31/1/75	Prednisone 15 mg & dapsone 100 mg	Active	1:80
20/3/75	Prednisone 15 mg & dapsone 100 mg	No	1:80
25/4/75	Same	No	1:80
23/5/75	Same	No	1:40
Case 3			
28/11/74	Prednisone 60 mg (Transfer to another hospital for appendicectomy 3/12/74–26/12/74)	Active	1:320
27/12/74	Prednisone 10 mg & dapsone 100 mg	Active	—
8/1/75	Same	No	1:80
23/5/75	Same	No	1:20

had no active lesions since. The titres dropped from 1:320 to 1:80 (Table 1). He was discharged on 21 January 1975 and continued the same dose without any side effects (May 1975).

DISCUSSION

Bystryn, Abel & De Feo (1974) could demonstrate subcorneal fluorescent intercellular antibodies in four of ten sera from patients with pemphigus foliaceus. Pemphigus antibodies were shown as a single line between the uppermost keratinocytes and the stratum corneum of normal human skin. It was concluded that the antigens reacting with the intercellular antibodies in pemphigus vulgaris are different from those binding subcorneal intercellular antibodies.

The findings in case 1 reported here were somewhat different. The fluorescent intercellular antibodies were present in the Malpighian and basal cell layers, not in the subcorneal layer, of normal human skin. The peroxidase-staining was present as an intercellular network only in the granular layer of hyperkeratotic human skin, not in the normal human skin even though consecutive cuts of the same substrate were used in both techniques. The pattern was similar to the fluorescent staining in the uppermost cellular spaces of guinea-pig oesophagus demonstrated by Bystryn *et al.* (1974), and was not present as a single line such as they found in the subcorneal layer of human epidermis. Looking carefully at the illustrations demonstrated by Fellner *et al.* (1973), one can also see evidence of peroxidase-intercellular antibodies due to the penicillin reaction in the upper layers of the hyperkeratotic epidermis of the guinea-pig lip. The localization of the peroxidase-intercellular antibodies in this report and in the penicillin reaction possibly represents the affinity of the peroxidase-conjugates for the intercellular antibodies in the upper layers of the epidermis. These findings suggest that patients with pemphigus foliaceus may have the same intercellular antibodies as in pemphigus vulgaris, but binding at different anatomical sites.

The second patient developed hepatotoxicity even though he was treated carefully with cyclophosphamide and was having special laboratory tests every week. Prednisone alone could not control the disease. Since so many patients suffer from the complications of cytotoxic drugs and systemic corticosteroids (Lever, 1972; Krain, 1974; Piamphongsant & Sivayathorn, 1975), an alternative treatment for this disease is desirable. Dapsone was chosen as it has been used effectively to control immunological disorders, such as classical dermatitis herpetiformis, pemphigoid (van der Meer, 1969; Piamphongsant *et al.*, 1975), and vasculitis (Cream, Levene & Calnan, 1971). It has also been effective in vesiculobullous eruptions, such as subcorneal pustular dermatosis (Sneddon–Wilkinson disease), generalized pustular psoriasis (MacMillan & Champion, 1973), and benign familial chronic pemphigus (Sire & Johnson, 1971). Similar cases have been successfully controlled by dapsone at our Institute. The mechanism of action in these diseases is not known but it could possibly act by inhibiting lysosomal enzymes (Barranco, 1974b; Mier & Hurk, 1975). However, dapsone should not be used routinely in all cases of pemphigus, since failures have also been observed at the Institute in other patients of the same young age group who had the same clinical severity and the same levels of intercellular antibodies.

It is possible that the patients in this report are a group distinct from patients with pemphigus who do not respond to dapsone. It would appear that some patients with pemphigus who respond to dapsone tend to be younger, although not all younger cases respond to dapsone. Also, the disease tends to run a more benign course.

At present, there is no reliable means of selecting cases for dapsone treatment. Whether it is possible to use the mistaken clinical impression of a double diagnosis with dermatitis herpetiformis, as in previous reported cases which responded to dapsone, is doubtful. In conclusion, this report emphasizes that vesiculo-bullous diseases which can be controlled by dapsone are not always dermatitis herpetiformis.

REFERENCES

BARRANCO, V.P. (1974a) Mixed bullous disease. *Archives of Dermatology*, **110**, 221.

BARRANCO, V.P. (1974b) Inhibition of lysosomal enzymes by dapsone. *Archives of Dermatology*, **110**, 563.

BEUTNER, E.H. CHORZELSKI, T.P. & JORDON, R.E. (1970) *Autosensitization in pemphigus and bullous pemphigoid.* Charles C. Thomas, Springfield.

BYSTRYN, J-C, ABEL, E. & DE FEO, C. (1974) Pemphigus foliaceus: subcorneal intercellular antibodies of unique specificity. *Archives of Dermatology*, **110**, 857.

CREAM, J.J., LEVENE, G.M. & CALNAN, C.D. (1971) Erythema elevatum diutinum: an unusual reaction to streptococcal antigen and response to dapsone. *British Journal of Dermatology*, **84**, 393.

DEMENTO, F.J. & GROVER, R.W. (1973) Acantholytic herpetiform dermatitis. *Archives of Dermatology*, **107**, 883.

DOEPFMER, R. VON (1961) Ueber eine nosologisch ungeklärte bullose Dermatose (Pemphigus chronicus vulgaris oder Dermatitis herpetiformis Duhring). *Hautarzt*, **12**, 452.

EMMERSON, R.W. & WILSON JONES, E. (1968) Eosinophilic spongiosis in pemphigus. *Archives of Dermatology*, **97**, 252.

FELLNER, M.J., FUKUYAMA, K., MOSHELL, A. & KLAUS, M.V. (1973) Intercellular antibodies in blood and epidermis. A histochemical study of IgG immunoglobulins in patients with late reactions to penicillins and their comparison with similar antibodies in patients with pemphigus vulgaris. *British Journal of Dermatology*, **89**, 115.

FLODEN, C.H. & GENTELE, H. (1955) A case of clinically typical dermatitis herpetiformis (Duhring) presenting acantholysis. *Acta dermato-venereologica*, **35**, 128.

MACMILLAN, A.L. & CHAMPION, R.H. (1973) Generalized pustular psoriasis treated with dapsone. *British Journal of Dermatology*, **88**, 183.

MEER, J.B. VAN DER (1969) Granular deposits of immunoglobulins in the skin of patients with dermatitis herpetiformis: An immunofluorescent study. *British Journal of Dermatology*, **81**, 493.

MIER, P.D. & VAN DEN HURK, J.J.M.A. (1975) Inhibition of lysosomal enzymes by dapsone. *British Journal of Dermatology*, **93**, 471.

KRAIN, L.S. (1974) Pemphigus: epidemiologic and survival characteristics of 59 patients, 1955–1973. *Archives of Dermatology*, **110**, 862.

LEVER, W.F. (1972) Methotrexate and prednisone in pemphigus vulgaris. *Archives of Dermatology*, **106**, 491.

PIAMPHONGSANT, T. & SIVAYATHORN, A. (1975) Pemphigus: combined treatment with methotrexate and prednisone. *Journal of the Medical Association of Thailand*, **58**, 172.

PIAMPHONGSANT, T., CHAIKITTISILPA, S., & KULLAVANIJAYA, P. (1975) *Juvenile Pemphigoid* (in press).

SEAH, P.P., FRY, L., LIONEL, F., CAIRNS, R.J. & FEIWEL, M. (1973) Pemphigus controlled by sulfapyridine. *British Journal of Dermatology*, **89**, 77.

SIRE, D.J. & JOHNSON, B.L. (1971) Benign familial chronic pemphigus treated with dapsone. *Archives of Dermatology*, **103**, 262.

WINKELMANN, R.K. & ROTH, H.L. (1960) Dermatitis herpetiformis with acantholysis or pemphigus with response to sulfonamides. *Archives of Dermatology*, **82**, 385.

British Journal of Dermatology (1976) **94**, 687.

The treatment of hypermelanosis with 4-isopropylcatechol

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SUMMARY

Over the past 6 years sixty-eight patients have been treated with 1% or 3% 4-isopropylcatechol (4-IPC). Fifty-four had melasma, the others a variety of disorders of pigmentation. Two-thirds of the patients were significantly improved. Twenty patients had skin irritation due to 4-IPC and four developed an allergic contact dermatitis. One patient developed confetti-like areas of depigmentation in the 4-IPC-treated areas.

Light and electron microscopic studies showed that in the 4-IPC-treated areas there was a loss and damage of the melanocytes, but the keratinocytes and Langerhans cells were unaffected. Melanosomal complexes containing many melanosomes were frequently found in the keratinocytes of the 4-IPC-treated negro skin.

4-Isopropylcatechol is a potent depigmenting agent and is of use in the topical therapy of selected patients with hypermelanosis. However, like other substituted phenols and hydroquinone it is irritant to the skin and should be used with caution.

At the present time the treatment of hypermelanosis of the skin is unsatisfactory. Various compounds, including hydroquinone and the monobenzyl and monomethyl ethers of hydroquinone, have been used topically to depigment the skin, but these are variable in their effect and often irritate the skin. Experimental studies have shown that a number of substituted phenols have a selective lethal effect on mammalian melanocytes both *in vivo* (Bleehen *et al.*, 1968; Riley, 1969; Frenk, 1970) and *in vitro* (Riley, 1970; Bleehen, 1975). Of a number of compounds tested for their depigmenting effect by the topical application to the skin of black guinea-pigs, 4-isopropylcatechol (4-IPC) was found to be the most potent (Bleehen *et al.*, 1968). This compound was more effective than the known depigmenting compounds hydroquinone and the monobenzyl and monomethyl ethers of hydroquinone.

The purpose of this paper is to report the clinical use over a period of 6 years of 1% and 3% 4-isopropylcatechol creams for the therapy of various hypermelanotic and other pigmentary disorders. Histological studies on biopsies of skin treated with 4-IPC indicate that this compound has a destructive effect on functional melanocytes.

PATIENTS AND METHODS

Sixty-eight patients with various pigmentary disorders (Table 1) were treated with 1% or 3% 4-IPC creams. The preparations were applied once or twice daily to selected affected areas. The patients were photographed in black and white and in colour before and during treatment. The assessment of therapy was aided by the use of these serial photographs. Patients were seen initially fortnightly and later on at monthly intervals. The majority had treatment for at least 3 months, some remaining on intermittent therapy for several years.

Shave biopsies of skin from both 4-IPC-treated and opposite untreated control areas were taken from three patients. One with melasma was treated for 3 days with 3% 4-IPC, the other two with normal pigmented skin were treated with 3% 4-IPC for 3 weeks. All three patients were African negroes. Each biopsy was divided into three portions. One portion was fixed in 10% formol saline and vertical sections of paraffin embedded skin were cut and then stained with haematoxylin and eosin as well as with a silver stain for melanin. One portion was incubated in 2 N NaBr at 37°C for 2 h and the separated epidermal sheet then incubated in 0·1% L dopa at pH 7·2 for 4 h according to the method of Staricco & Pinkus (1957). The third portion was fixed in 3% glutaraldehyde and after embedding in araldite, ultrathin sections were cut and stained and later examined in the electron microscope.

TABLE 1. Disorders of pigmentation treated with 4-IPC

Disorder	No. of patients treated	No. improved
Melasma	54	42
Post-inflammatory hyperpigmentation	6	2
Vitiligo	4	2
Pigmented naevi	2	0
Lentigines	2	0

RESULTS

Clinical findings

The results of treatment with 1% and 3% 4-IPC are shown in Table 1. The majority of patients treated had melasma. These patients were mainly female, many of them being young and on the contraceptive pill, and usually with a marked hypermelanosis of the cheeks and forehead. About 80% of these patients were improved and most of them within 1 month of treatment (Figs 1 and 2). In the 4-IPC treated areas not only was the excess pigment removed, but in coloured patients there was also a lightening of the colour of the skin (Fig. 3). The rate of removal of the excess pigment was very variable. In some the depigmentation of the skin was noted within 3 weeks of the start of treatment, but in some it took as long as 2 months. Treatment with 3% 4-IPC was more effective than 1% 4-IPC in that it produced earlier detectable depigmentation of the skin, but this higher concentration was more irritant to the skin and side effects were more frequent. Many patients who started

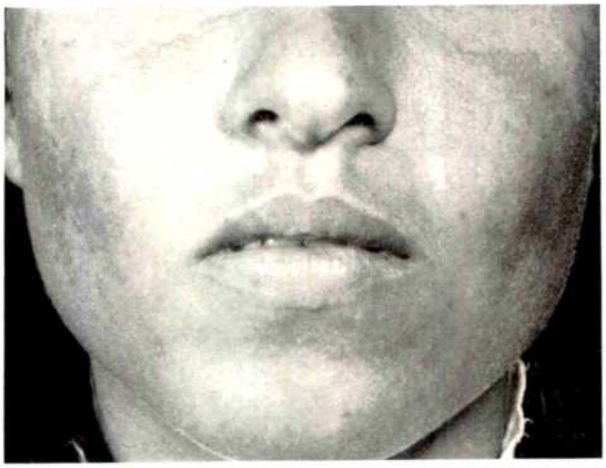

FIGURE 1. Melasma prior to treatment.

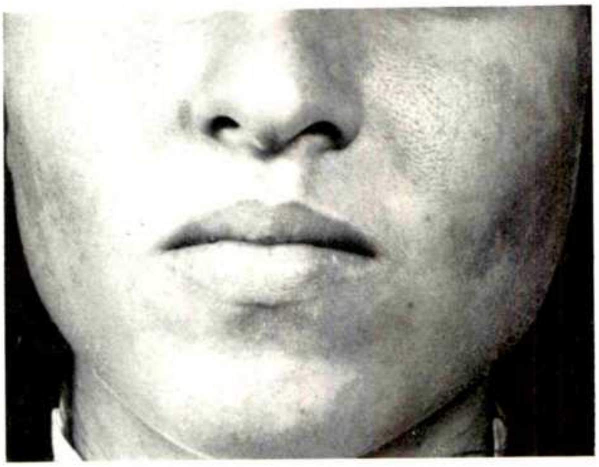

FIGURE 2.

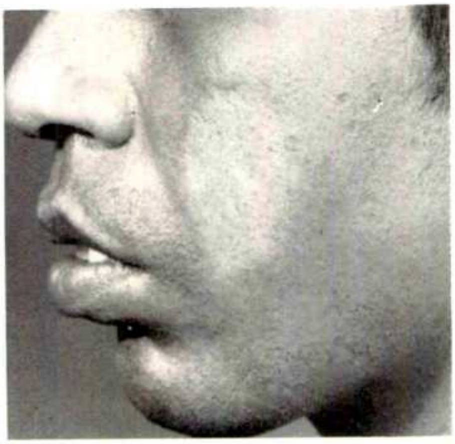

FIGURE 3.

FIGURE 2. Same patient after 1 month of treatment with 3% 4-IPC to right side of face and a control cream to the left side. Much of the excess pigment on the right side of face has been removed.

FIGURE 3. Depigmentation of skin on cheek of an Asian treated with 3% 4-IPC for 3 weeks. This area repigmented after stopping treatment.

with 3% 4-IPC were later changed during the trial to 1% 4-IPC. The patients with melasma responded very well to treatment with 4-IPC which they found far more effective than 5% hydroquinone. This group was highly delighted with the cosmetic results of treatment.

Of six patients with post-inflammatory hyperpigmentation, including one with an extensive berloque dermatitis, only two showed any improvement with 4-IPC. Two of four patients with almost universal vitiligo responded to treatment with bleaching away of residual hypermelanotic areas on the face and neck. However, treatment had to be discontinued in one after 6 weeks of treatment, as

TABLE 2. Occlusive patch tests to 4-isopropylcatechol (4-IPC)

20 treated patients		50 normals	
% concentration 4-IPC	No. positive	% concentration	No. positive
0·1	2	1	2
0·5	4	3	4
1·0	4	5	7

TABLE 3. Effect of 3 weeks treatment with 3% 4-IPC on melanocyte population of facial skin

	Melanocytes/mm² ± s.e. mean	
Patient	Control	4-IPC treated skin
A.D.	1360 ± 80	980 ± 50
P.M.	1940 ± 65	650 ± 85

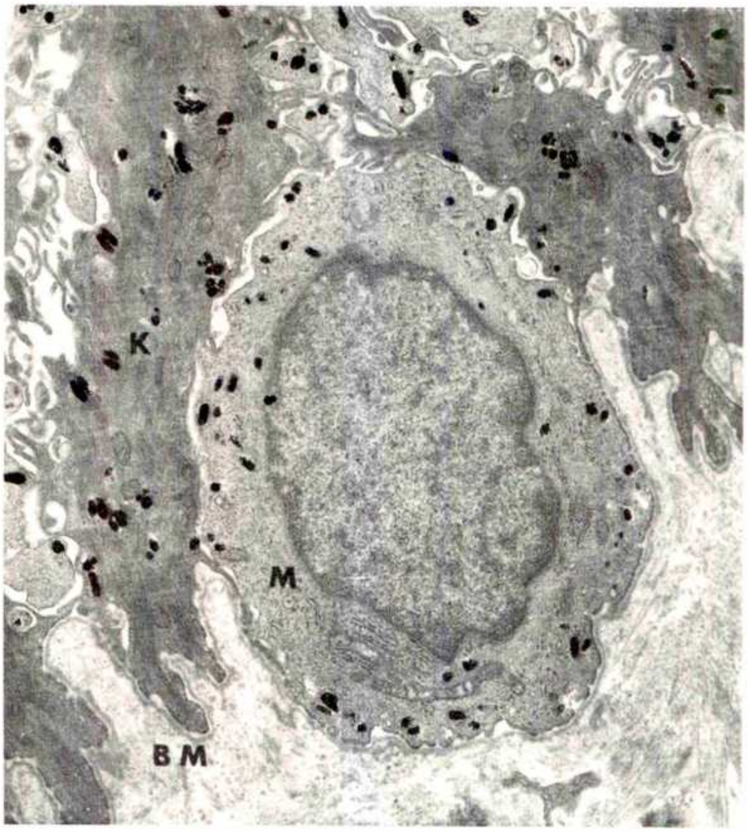

FIGURE 4. Electron micrograph of basal layer of normal negro skin. Melanocyte (M), Keratinocytes (K) and Basement membrane (BM). (Magnification × 8000.)

she developed a contact dermatitis. The patient had previously had an allergic contact dermatitis to the monobenzyl ether of hydroquinone.

On stopping treatment, many patients, particularly those with melasma, noted that the hypermelanosis recurred over a period of several months. This was particularly noticeable during the summer with sun exposure. Some patients have found that using 1% 4-IPC cream once or twice weekly has been sufficient to keep away the excess pigment.

Side effects

Of the sixty-eight patients in this trial, twenty noted side effects. In many, these were quite trivial and did not necessitate stopping treatment. The most common symptom was that patients noted, soon after applying the 4-IPC cream, some tingling of the treated skin, particularly early on in therapy. In a number, redness and scaling of the skin developed, especially in those using 3% 4-IPC. Four of these patients had an allergic contact dermatitis which was subsequently proved by patch tests. The results of patch tests with 0·1%, 0·5% and 1% 4-IPC in twenty patients with symptoms of skin irritation are shown in Table 2. Eight of these patients were also tested with 1% 4-*n*-propylcatechol, 1% monobenzyl ether of hydroquinone, 1% hydroquinone and with a parabens mixture. Of these, two gave positive patch tests to 4-*n*-propylcatechol and one to monobenzyl ether of hydroquinone and all three gave positive patch tests to 4-IPC.

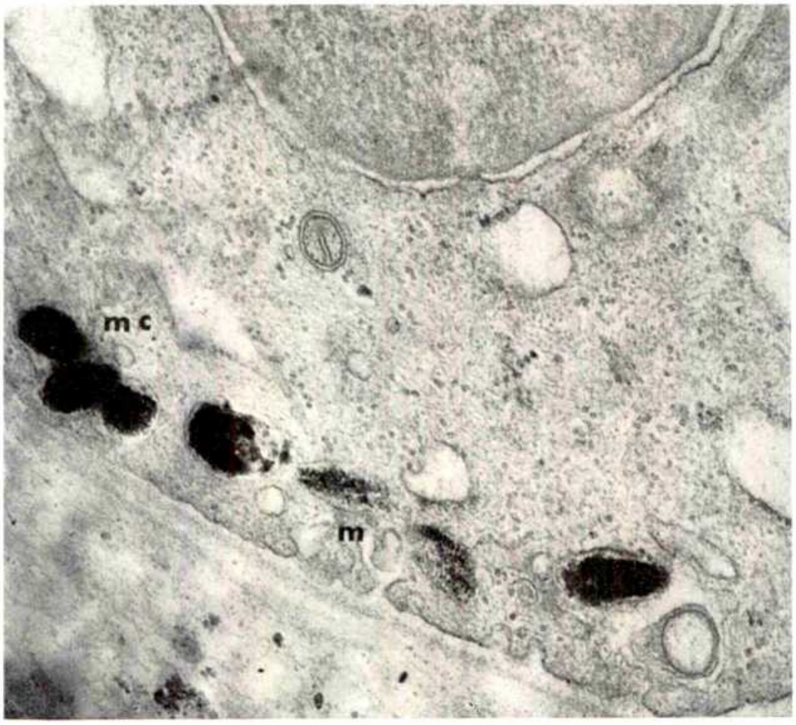

FIGURE 5. Portion of a melanocyte in biopsy of skin treated with 3% 4-IPC for 3 weeks. There is considerable swelling of the cisternae of rough endoplasmic reticulum and of the nuclear membrane and distortion of the outer membranes of melanosomes (m). A melanosome complex (mc) is present in this damaged melanocyte. (Magnification × 40,000.)

Occlusive patch tests were also carried out in fifty normal patients with 1%, 3% and 5% 4-IPC. The results of these patch tests read at 48 h are shown in Table 2.

One patient, an Asian male with hyperpigmentation of his cheeks, developed, after 5 months of treatment, confetti-like areas of depigmentation in the 4-IPC-treated areas. These areas did not progress and were confined to the face. Four months after stopping treatment, these confetti-like areas of depigmentation were still present and had only partially repigmented.

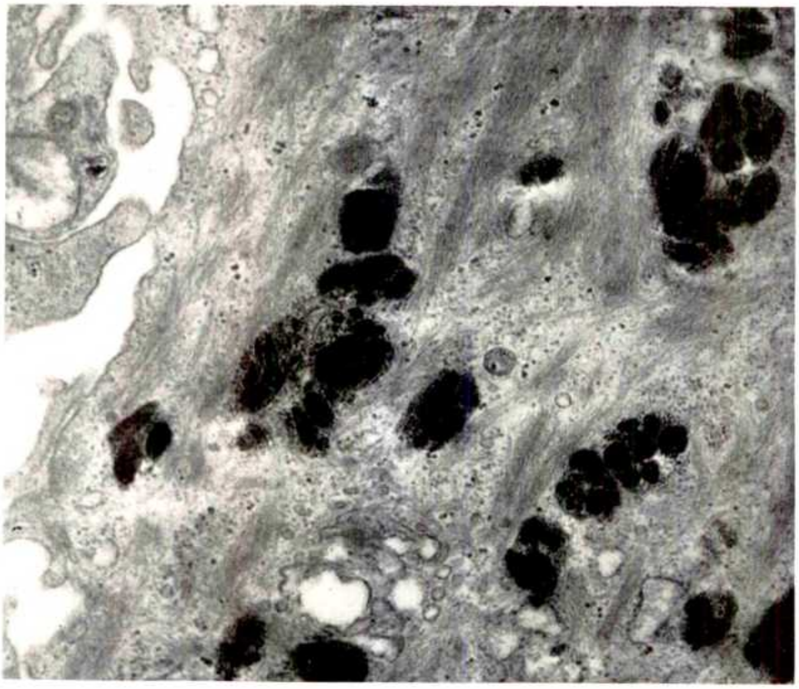

FIGURE 6. Melanosomes in negro skin treated for 3 weeks with 3% 4-IPC mainly aggregated together as melanosomal complexes in keratinocyte. (Magnification × 32,000.)

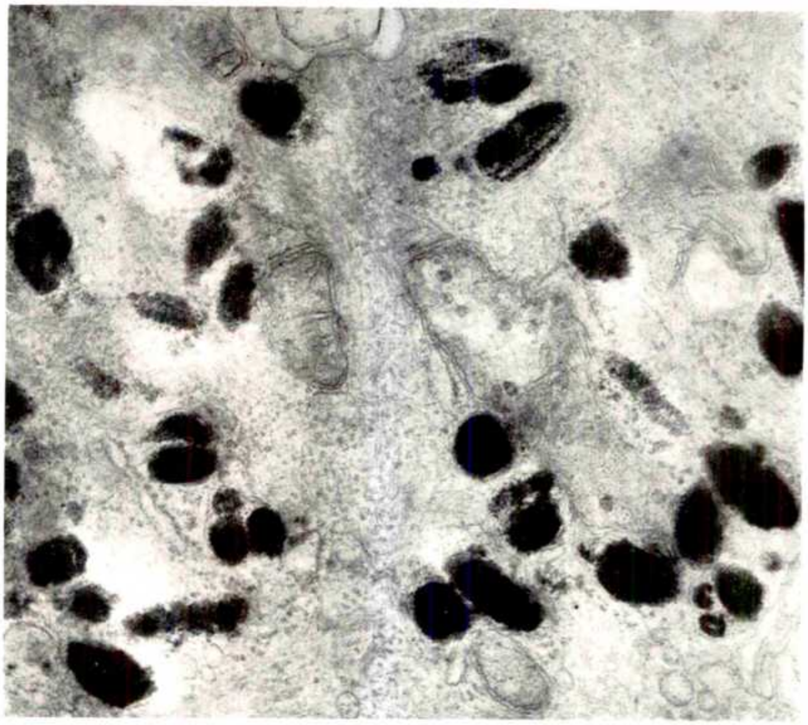

FIGURE 7. Melanosomes in keratinocyte of normal untreated negro skin. Ellipsoidal or rounded electron dense melanosomes mainly arranged singly with a few melanosomal complexes. (Magnification × 40,000.)

Histological findings

Vertical sections of 4-IPC-treated skin when stained for melanin showed a reduction in the amount of melanin when compared with control untreated areas.

Dopa-incubated epidermal sheets of biopsies of skin taken from two patients treated for 3 weeks with 3% 4-IPC to one side of the forehead showed a reduction in the population density of functional melanocytes when compared with those from the control untreated side of the forehead (Table 3).

Electron microscopic studies of skin treated with 4-IPC showed a disruption of the normal subcellular architecture of many of the melanocytes lying in the basal layer of the epidermis (Fig. 4). Two types of damaged melanocytes were seen: one type, in which damage was minimal, contained partially melanized melanosomes, but also showed myelin-like bodies; the other type, particularly seen in skin treated with 4-IPC for longer periods, was a lethally damaged cell with swelling and vacuolization of membranous organelles. The nuclear membrane, golgi and cisternae of rough endoplasmic reticulum seemed particularly affected as well as the outer limiting membrane of the melanosomes which was also frequently distorted (Fig. 5). Melanosomal complexes were not infrequently found in the damaged melanocytes (Fig. 5). The Langerhans cells were unaffected and were found in the basal layer as well as higher up in the epidermis. Indeterminate dendritic cells were also present but these were considered to be Langerhans cells without the characteristic organelles rather than non-melanogenic melanocytes.

In the Negro skin treated with 4-IPC for 3 weeks the melanosomes in the keratinocytes tended to be aggregated to form melanosomal complexes (Fig. 6) often containing five or more melanosomes. In the control untreated skin these melanosomes in the keratinocyte were larger and were usually arranged either singly or as melanosomal complexes containing two to three melanosomes (Fig. 7).

The basement membrane in the sections examined appeared intact and there was no basal cell encroachment into the dermis. A few melanophages were found in the dermis of the 4-IPC-treated skin.

DISCUSSION

4-Isopropylcatechol is a most effective compound for the therapy of hypermelanosis in man. This compound is more potent and more consistent in its depigmenting effect than hydroquinone or the monobenzyl ether of hydroquinone. The ineffectiveness of these compounds led to the clinical use of 4-IPC. Previous personal experience using 2% and 5% hydroquinone creams for the therapy of melasma has been disappointing and differs from the good results obtained by other workers (Spencer, 1961; Fitzpatrick *et al.*, 1966). 4-IPC like other substituted phenols and hydroquinone is a primary irritant and in this trial the incidence of side effects was high. It is a contact sensitizer like the monobenzyl ether of hydroquinone and in this trial, four of sixty-eight patients developed an allergic contact dermatitis after varying lengths of treatment. Perhaps the most disturbing side effect is that one patient developed confetti-like depigmented macules in the 4-IPC-treated areas on the face, such as is not infrequently seen with the use of the monobenzyl ether of hydroquinone (Dorsey, 1960). Other melanocidal compounds, such as p-tertiary amyl phenol and p-tertiary butyl phenol, can also produce an irreversible depigmentation of the skin (Kahn, 1970).

The use of 4-IPC is only advocated for the therapy of selected patients who are under regular supervision and in whom other depigmenting preparations such as hydroquinone have proved to be ineffective. Perhaps the new formulation of hydroquinone (Kligman & Willis, 1975) will prove to be more useful for the therapy of hypermelanotic disorders.

Histological studies have shown that 4-IPC has a specific melanocytotoxic effect and that cutaneous depigmentation results from a loss of functional melanocytes. The probable mode of action is that this compound acts as a substrate for tyrosinase and is oxidized to form a toxic free radical derivative. This free radical induces a chain reaction of lipid peroxidation and results in the destruction of the lipoprotein membranes of the cell (Riley, 1970; Copeman, Lewis & Bleehen, 1973).

In vitro studies indicate that 4-IPC has a rapid lethal effect on pigmented guinea-pig melanocytes when used in a concentration 10^{-3}M (Riley, 1970). Similar studies using Harding-Passey, B16 and human melanoma cells have shown that 4-IPC in this concentration is toxic, especially to the more pigmented cells (Bleehen, 1975). Ultrastructural observations on melanoma cells exposed for 30–60 min showed that there was considerable disruption of the sub-cellular architecture with distortion and swelling of membranous organelles (Bleehen, 1975). Similar changes were seen in the melanocytes of skin treated with 4-IPC even after only 3 days of therapy.

The aggregation of melanosomes in the keratinocytes of Negro skin treated with 4-IPC and the formation of large melanosomal complexes are similar to what has been observed in guinea-pig skin treated with hydroquinone (Jimbow *et al.*, 1974). It is likely that the damaged outer limiting membrane of the melanosomes allows it to fuse with that of other melanosomes and that this may occur within the melanocyte or after the transfer of the melanosomes to the keratinocyte.

4-Isopropylcatechol is a most potent depigmenting compound and is effective in the treatment of various hypermelanotic conditions when previous therapy with hydroquinone has failed. However, it should be used with caution since like other substituted phenols it is an irritant and a contact sensitizer.

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REFERENCES

Bleehen, S.S., Pathak, M.A., Hori, Y. & Fitzpatrick, T.B. (1968) Depigmentation of skin with 4-isopropylcatechol, mercaptoamines and other compounds. *Journal of Investigative Dermatology*, **50**, 103.

Bleehen, S.S. (1975) Selective lethal effect of substituted phenols on cell cultures of malignant melanocytes. In: *Proceedings of 9th International Pigment Cell Conference* (Ed. by V. Riley). Karger, Berlin (In press).

Copeman, P.W.M., Lewis, M.G. & Bleehen, S.S. (1973) Biology and immunology of vitiligo and cutaneous malignant melanoma. In: *Recent Advances in Dermatology* (Ed. by A. Rook), p. 257. Churchill Livingstone, Edinburgh.

Dorsey, C.S. (1960) Dermatitis and pigmentary reactions to monobenzyl ether of hydroquinone. *Archives of Dermatology*, **81**, 245.

Fitzpatrick, T.B., Arndt, K.A., El Mofty, A.M. & Pathak, M.A. (1966) Hydroquinone and psoralens in the therapy of hypermelanosis and vitiligo. *Archives of Dermatology*, **93**, 589.

Frenk, E. (1970) Dépigmentation expérimentale de la peau du cobaye noir. *Annales de Dermatologie et de Syphiligraphie*, **97**, 287.

Jimbow, K., Obata, H., Pathak, M.A. & Fitzpatrick, T.B. (1974) Mechanism of depigmentation by hydroquinone. *Journal of Investigative Dermatology*, **62**, 436.

Kahn, G. (1970) Depigmentation caused by phenolic detergent germicides. *Archives of Dermatology*, **102**, 77.

Kligman, A.M. & Willis, I. (1975) A new formula for depigmenting human skin. *Archives of Dermatology*, **111**, 40.

Riley, P.A. (1969) Hydroxyanisole depigmentation *in vivo* studies. *Journal of Pathology*, **97**, 185.

Riley, P.A. (1970) Mechanism of pigment-cell toxicity produced by hydroxyanisole. *Journal of Pathology*, **101**, 163.

Spencer, M.C. (1961) Hydroquinone bleaching. *Archives of Dermatology*, **84**, 131.

Staricco, R.J. & Pinkus, H. (1957) Quantitative and qualitative data on the pigment cells of adult human epidermis. *Journal of Investigative Dermatology*, **28**, 33.

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Case Report

Hodgkin's disease and pemphigus

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SUMMARY

The second case of pemphigus associated with Hodgkin's disease is reported. Circulating pemphigus auto-antibodies were detected in this patient and cell-mediated (T cell) immunity was markedly depressed. It is postulated that this T-cell immunodepression predisposed the patient to the development of pemphigus.

The association between pemphigus and Hodgkin's disease has only once been recorded (Sood & Pasricha, 1974). We report and discuss a patient with disseminated Hodgkin's disease (with marked depression of cellular immunity) and pemphigus foliaceus.

CASE REPORT

A 52-year-old female presented with a 6-month history of pruritus, night sweats and weight loss. On examination, there was gross generalized lymphadenopathy and hepatosplenomegaly; a fading desquamating macular eruption was noted on her buttocks and thighs. Lymph node biopsy showed Hodgkin's lymphoma (lymphocyte depletion type). The patient was stage 3B (Ann Arbor classification). During investigation she developed a painful bullous erythematous skin eruption on her buttocks, thighs and lower abdomen. Swabs taken from the lesions for bacterial culture were initially sterile; later *Staphylococcus aureus* infection supervened. Skin biopsy of an early lesion showed a subcorneal blister containing a few neutrophil polymorphs. There was acantholysis in the floor of the lesion (Fig. 1). A diagnosis of pemphigus foliaceus was made.

Immunological investigation showed depressed cellular immunity as shown by *in vitro* lymphocyte studies and by anergy to five skin test-recall antigens. The patient was lymphopenic (800/mm^3). Serum immunoglobulins were IgG 8·64, IgA 0·84, IgM 0·72 g/L. Serum smooth muscle, thyroid and anti-mitochondrial antibodies, anti-nuclear and rheumatoid factors were not detected. Circulating pemphigus antibodies were detected to a titre of 1–40 dilution.

The patient was treated with quadruple cytotoxic chemotherapy (including prednisolone 40 mg daily) and skin lesions were treated locally with clobetasol propionate 0·05% ointment (Dermovate, Glaxo). The skin lesions cleared over the next 3–4 weeks and her lymphadenopathy and hepatosplenomegaly also resolved. Transient anuria (as a result of urate nephropathy) occurred soon after the start of chemotherapy; allopurinol therapy was added with good effect.

DISCUSSION

It is thought that B-cell capacity to produce antibodies against exogenous antigens and against the individual's own tissues is kept in check by thymus-dependent lymphocytes (T cells) (Allison,

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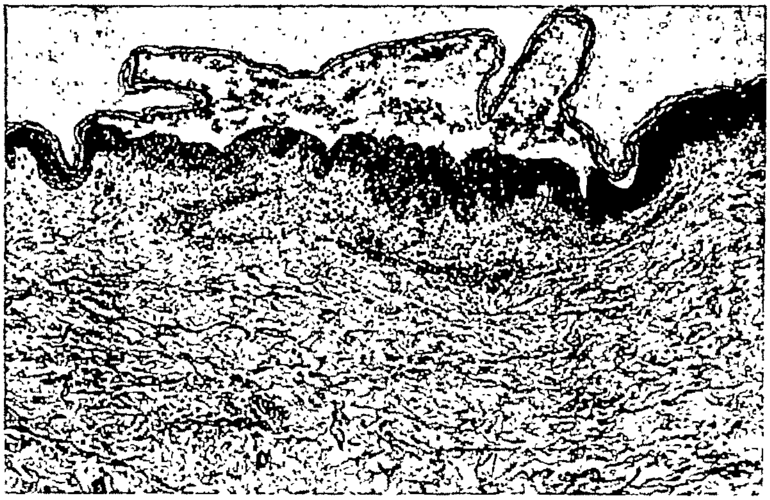

FIGURE I.

Denman & Barnes, 1971). This implies that auto-antibodies may develop in patients with depressed cellular immunity. Certainly up to 20% of patients with lymphomas (Hancock, unpublished work) have depression of cellular immunity and this could account for the reported incidence of associated auto-immune disturbance particularly haemolytic anaemia (Rosenthal *et al.*, 1955; Miller, 1967.) In Hodgkin's disease immunity is more depressed with advanced stages of disease and with lymphocyte-depleted histology (Young *et al.*, 1972).

Pemphigus is generally recognized to be an auto-immune disease but two cases have been reported in association with lymphoreticular malignancy (Saikia, 1972; Sood & Pasricha, 1974). In the first of these cases a giant follicular lymphoma tumour was found at autopsy to be producing large quantities of pemphigus antibodies. Circulating pemphigus auto-antibodies were found in our patient's serum. She had disseminated Hodgkin's disease and her cell-mediated immunity was depressed as shown by both *in vitro* and *in vivo* assessment. She was also lymphopenic with lymphocyte-depleted histology. Such marked immunodepression could certainly be implicated in a causal relationship between her Hodgkin's disease and pemphigus.

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REFERENCES

ALLISON, A.C., DENMAN, A.M. & BARNES, R.D. (1971) Co-operating and controlling functions of thymus-derived lymphocytes in relationship to auto-immunity. *Lancet*, **ii**, 135.

MILLER, D.G. (1967) The association of immune disease and malignant lymphoma. *Annals of Internal Medicine*, **66**, 507.

ROSENTHAL, M.C., PISCIOTTA, A.V., KOMMINOS, Z.D., GOLDENBERG, H. & DAMESHEK, W. (1955) The auto-immune haemolytic anaemia of malignant lymphocytic disease. *Blood*, **10**, 197.

SAIKIA, N.K. (1972) Extraction of pemphigus antibodies from a lymphoid neoplasm and its possible relationship to pemphigus vulgaris. *British Journal of Dermatology*, **86**, 411.

SOOD, V.D. & PASRICHA, J.S. (1974) Pemphigus and Hodgkin's disease. *British Journal of Dermatology*, **90**, 575.

YOUNG, R.C., CORDER, M.P., HAYNES, H.A. & DE VITA, V.T. (1972) Delayed hypersensitivity in Hodgkin's disease. *American Journal of Medicine*, **52**, 63.

British Journal of Dermatology (1976) **94**, 697.

Comment

NAILS AND FUNGI

Human nails support a surprisingly varied fungal flora, including not only dermatophytes and yeasts, but many mould species both common and uncommon. These infections are known by two names, onychomycosis and tinea unguium. Onychomycosis literally means 'fungus infection of the nail', while 'tinea' is an ancient word for 'ringworm'. I propose, here, to use 'onychomycosis' to include nail infections attributable to any fungus, and to confine 'tinea unguium' to dermatophyte infections only.

Laboratory criteria for a diagnosis of onychomycosis

Moulds, the spores of which are abundant in the air, and to a lesser extent yeasts which may exist on the patient's skin, can be isolated from many nails on actidione-free media. It is important, when diagnosing onychomycosis, to distinguish between contaminants from these sources and saprophytic fungi genuinely growing in the nail. In my investigations I have adopted the following criteria, which must have been fulfilled before a diagnosis of onychomycosis due to a particular mould or yeast is accepted. (1) If a dermatophyte is isolated it is taken to be the pathogen without supporting evidence. (2) If a mould or yeast is isolated it is only considered of significance if the appropriate fungal elements —mycelium, arthrospores or yeast cells—are seen on direct microscopy of the nail. (3) For a diagnosis of mould infection, no dermatophyte must have been isolated on actidione-containing or actidione-free medium, and at least 5/20 inocula must have yielded the mould.

Isolation of fungi from infected nails

The difficulty of isolating fungi from microscopically positive nails is well known (Gentles, 1971; English & Lewis, 1974; Zaias, 1972) and some reasons will be discussed later. Because of this poor isolation rate any attempt to assess the relative incidence of the different fungi which can cause onychomycosis is very unsatisfactory. Various methods have been used to overcome the difficulty. An ingenious grinder for powdering nail clippings was described by Zaias, Oertel & Elliott (1969) but I have not had any great success with it, probably because the sample used consists only of the usual clipping from the distal end of the nail. A more satisfactory method would, theoretically, be to collect material from all over the nail plate and for some depth into it. This can be done by using for sampling a dental drill with a suction nozzle attached, by means of which the nail dust generated by the drill is collected into a bag and can be used for microscopy and culture. I have found such a technique to be very effective in practice, and to raise the success rate in culturing from microscopically positive nails to about 88% from the usual 50–75% (English & Atkinson, 1973). The use of the method should give a more reliable measure of the incidence of the various types of fungal infection of nails than has so far been available.

The nail plate as substrate

To a fungus the nail plate would seem to be a very inhospitable habitat, being dry, indigestible and lacking in nutrients (Marples, 1965). The outer surface and distal end have, however, one advantage; they are far removed from the body's defence mechanisms. Other parts are closely integrated with, or spring from, the nail bed and nail fold and are therefore in closer contact with living cells and more likely to be affected by their activities. But these parts will have a higher moisture content, more nutrients, and consist of softer and therefore more easily digested keratin than the outer surface and nail tip. Fungi colonizing nails, therefore, must be able to adapt either to fairly close contact with the living host, or to extremes of desiccation and diet. The differing climates in which finger- and toe-nails exist provide another variable, at least in shoe-wearing civilizations; that of toe-nails, kept warm and moist in encasing footwear, would be less rigorous than that of finger-nails. And pre-existing disease or trauma may profoundly affect the chemistry of the keratin and therefore the ease with which it may be broken down. On the interplay of all these factors will depend which fungus is able to colonize any particular nail or part of a nail and the form in which it will grow there, for different species have different climatic and nutritional requirements and are affected differently by proximity to the living body tissues.

Three groups of fungi are involved in onychomycosis: the dermatophytes, the moulds and the yeasts. Each group has characteristic growth requirements and limiting factors which differ from those of other groups; within each group some individual fungus species may have more or less specific requirements than others. It is to some extent possible to explain the clinical behaviour of fungi causing onychomycosis by relating these requirements and limitations to the varying habitats offered by different nails and different parts of the same nail.

The dermatophytes

The dermatophytes are universally accepted as primary pathogens of previously healthy skin, nail and hair. It has been shown by many workers, by means of both microscopic observation and chemical tests, that they can digest keratin *in vitro*, and this ability is usually considered to account for their unique habit as pathogens, for it has been taken for granted that they utilize these powers of keratinolysis *in vivo*. But Raubitshek (1961) suggested that dermatophytes on the host grow in and digest the intercellular cement, not the keratin, causing the clinical signs of scaling and of breaking up of hairs and nails by mechanically breaking apart the keratinized cells rather than by digesting them. Scanning electron microscope studies of parasitized hairs would seem to confirm this (Pucinelli & Ceccarcelli, 1969), but Sowinski's (1971) light microscope studies of tinea unguium show tunnels around hyphae in nail that could only be the result of digestion. This problem needs resolution as, if the undoubted keratinolytic abilities of the dermatophytes are not utilized in the parasitic phase, it is difficult to explain the unique position of these fungi as primary pathogens of keratinized tissues.

The dermatophytes most commonly associated with tinea unguium in Europe and North America are *Trichophyton interdigitale* (*T. mentagrophytes* var. *interdigitale*) and *T. rubrum*. *T. interdigitale* is normally confined to the feet and toe-nails, only rarely spreading to other parts of the body. *T. rubrum* in contrast, spreads readily, if slowly, to all parts of the body including the hands and finger-nails, and can therefore be considered to be a better adapted and more virulent parasite than *T. interdigitale*. That *T. interdigitale* is so rarely found in finger-nails could be due to the more rigorous environmental conditions provided by these nails than by toe-nails, but must also be associated with the fact that the fungus is so rarely a cause of tinea manuum that a reservoir of infection adjacent to these nails is lacking.

In toe-nails, which are freely attacked by both species, both can produce the typical clinical picture of tinea unguium, growing in the inner layers of the nail and usually associated with skin lesions. But *T. interdigitale* can also cause leukonychia trichophytica (Jessner, 1922) a strictly surface growth resulting in white patches on the nail. Such lesions are rarely associated with skin infection, and are typical of those caused by the moulds to be discussed later. It would seem, therefore, that *T. interdigitale* may be less well adapted to parasitism than is *T. rubrum* (Walshe & English, 1966), may sometimes show a preference for a habitat isolated from living cells, and has a greater ability than *T. rubrum* to colonize the very hard keratin found in such situations; that is, it behaves on occasion in very much the same way as the moulds.

Most dermatophyte species invade the middle and ventral layers of the nail plate adjacent to the bed, where the keratin is comparatively soft but is in close proximity to the living cells below. The nail bed itself is probably, in fact, the primary invasion site, and acts as a reservoir for continual reinfection of the growing nail. It is well known that, though a dermatophyte growing in skin is normally confined to the cornified layer and is not in direct contact with living cells, yet their mere proximity has a profound effect on the morphology of the fungus: the reproductive spores, or conidia, produced in culture, are invariably suppressed in the parasitic phase. In nail these fungi grow for preference in the softer keratin where, as in skin, they are adjacent to the living host tissues. And as in skin, though they are able to grow in this situation, their capacity to reproduce by means of conidia is suppressed; there is a very delicate balance between host and parasite. We know from the high failure rate of attempts to culture from nail clippings that the fungus is often dead by the time it reaches the distal end of the nail. This could be due to its inability to survive in keratin that is chemically very much harder than that of the inner and middle nail plate which is its preferred habitat, or possibly to loss of nutrients or moisture so far from the nail bed. We need much more knowledge of the physiology of these fungi before attempting an explanation.

Moulds

There is an ever-growing list of moulds which have been found actively colonizing nails. It includes *Hendersonula toruloidea* (Gentles & Evans, 1970; Campbell, 1974) which behaves in much the same way as a dermatophyte and colonizes skin as well as nails; *Scopulariopsis brevicaulis*, a mould which many would consider a primary pathogen of nails; the comparatively common *Aspergillus versicolor*, *A. terreus* and *Acremonium* (*Cephalosporium*) spp.; and also organisms such as the newly described *Pyrenochaeta unguium-hominis* whose only known habitat at present is the toe-nails (Punithalingham & English, 1975). None of these fungi is keratinolytic. Therefore all must either live on the unkeratinized intercellular cement, or must take advantage of partial denaturing of the nail keratin by pre-existing trauma or disease. It seems to be generally agreed that the latter is often the case and that, unlike the dermatophytes, the moulds with the exception of *H. toruloidea* and possibly *S. brevicaulis** are secondary invaders, never primary parasites. For instance, Walshe & English (1966) have shown that there is a comparatively high incidence of onychomycosis in patients with those skin diseases which can affect nails, and also in elderly people whose nails are often affected by the changes of senility. The nail keratin would be altered in both these groups. English & Atkinson (1974), surveying elderly chiropody patients, found that, of those with onychomycosis, double the proportion (25%) of infections were due to moulds as to dermatophytes (12%). This again would be compatible with nail changes associated with age allowing easy access to the moulds. Unlike dermatophytes, moulds usually colonize the outer layers of the nail, where they may have to combat extremes of desiccation and starvation, but are situated as far as possible from living host tissues. Leukonychia trichophytica (white patches on the extreme surface of the nail) is a common manifestation of mould infection. But moulds appreciate gentler environmental conditions should they be available. This is demonstrated by the case of English, Harman & Turvey (1967) where, in a patient with slightly deformed toes, *Cephalosporium* sp. was found colonizing only those toe-nails which were permanently overlapped, and therefore kept moist, by the adjacent toe.

As with dermatophyte infections, mould infections are much more common in toe-nails than in finger-nails. Here, there is no question of the adjacent skin as a source of the fungus at either site; the differing environmental conditions must alone account for the preference. For instance, in the case of finger-nails, usually only one nail is affected and a history of traumatic injury to this nail can generally be elicited. In contrast, it is not uncommon for several or even all toe-nails to be affected by the same mould. When it is remembered that all toe-nails are encased in the uniformly equable environment provided by footwear, and that if the patient suffers from a skin disease not due to fungi, or is elderly, all his toe-nails are likely to be affected rather than just one, this too becomes explicable.

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* *H. toruloidea* and *S. brevicaulis* are excluded from the following discussion as their behaviour *in vivo* differs so much from that of other moulds. Detailed physiological studies will be necessary to establish the reasons for this.

It has been noted that the conidia, or reproductive spores, of the dermatophytes are always suppressed in the parasitic phase. Here some moulds differ. *S. brevicaulis* and most *Aspergillus* spp. are capable of sporing *in vivo* and the spores can be seen on direct microscopy of the nail. This suggests that these fungi are growing as true saprophytes in the nail, unaffected by close proximity to living tissues. That they are not always found to be sporing could be due to a variety of reasons, including the particular cultural conditions in the nail at the time, or even to some ability to adapt to a parasitic phase.

All dermatophytes and moulds produce frond-like mycelium when growing *in vitro* on hard, layered substrata such as nail (English, 1963, 1965). These fronds seem to assist the fungus to grow between the layers of hard keratin, presumably digesting the intercellular cement as they grow. *In vivo* the production of this type of mycelium seems to be related to the degree to which the fungus can adapt to parasitism. Fronds are very rarely found *in vivo* when the causal fungus is *T. rubrum*, but are usually present if a mould is implicated (Zaias, 1972). *Trichophyton interdigitale*, the dermatophyte which as we have seen is capable of mould-like behaviour, may or may not produce fronds.

The yeasts

The ecology of the yeasts is very different from that of both the dermatophytes and the moulds. Many species of *Candida* and of yeasts belonging to other genera are found in nails but most are rarely seen on direct microscopy and appear to grow in the nail tissue strictly as saprophytes, doing no damage. Only *Candida albicans* and *C. parapsilosis*, the two species most frequently found in nails, can be considered as potential pathogens, and only they will be considered here.

Neither yeast is keratinolytic (English, 1965) and neither can colonize healthy nails. *C. albicans*, however, is usually considered to be an active pathogen while *C. parapsilosis* is not. Unlike the moulds, which may occur secondarily to any primary cause of damage to the nail, *C. albicans* is usually secondary only to chronic paronychia, the diseased nail bed and fold not only giving rise to a damaged nail but housing the inoculum of yeast ready to invade that nail (Whittle & Gresham, 1963), which it does in both the yeast and mycelial forms. Because chronic paronychia is normally, in the West at least, confined to the finger-nails, so is onychomycosis due to *C. albicans*. *C. parapsilosis* on the other hand is not usually associated with chronic paronychia and is generally found in the toe-nails. It is a common inhabitant of the normal skin (Böhme, 1965; Krasbach & Helige, 1964) which presumably serves as a reservoir of infection for the nails. This yeast, which forms mycelium readily *in vitro*, very rarely does so *in vivo* and this, together with the fact that it prefers the comparatively sheltered environment of toe-nails to the more rigorous one of finger-nails, is compatible with its being a weaker pathogen than *C. albicans*.

CONCLUSION

An attempt has been made to account for the various clinical manifestations of onychomycosis by considering, from the point of view of the fungi, the varying substrates offered by human nails. It is, perhaps, a 'fungus-eye view' of nails as possible ecological niches where the organism can obtain the environment and nutrients necessary to its survival. Despite their apparently inhospitable nature, nails offer hospitality to a wide range of fungi with very different physiological requirements and degrees of adaptation to growth on a living host. An attempt has been made to analyse the distinctive conditions offered by finger-nails as compared with toe-nails, by altered as compared with normal nails, and by different parts of the same nail, which would provide these varying conditions, each suited to the specific fungus or range of fungi normally found in them. Rather than coming to any definite conclusions a new way of looking at onychomycosis is offered, suggesting lines of research into the physiology of the fungi and the microstructure, microclimate and chemistry of nails which might help to elucidate the problems of onychomycosis.

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REFERENCES

BÖHME, H. (1965) Die Sprosspilzbesiedlung der Haut. *Dermatologische Wochenschrift*, **151**, 107.

CAMPBELL, C.K. (1974) Studies on *Hendersonula toruloidea* isolated from human skin and nail. *Sabouraudia*, **12**, 150.

ENGLISH, M.P. (1963) The saprophytic growth of keratinophilic fungi on keratin. *Sabouraudia*, **2**, 115.

ENGLISH, M.P. (1965) The saprophytic growth of non-keratinophilic fungi on keratinized substrata and a comparison with keratinophilic fungi. *Transactions of the British Mycological Society*, **48**, 219.

ENGLISH, M.P. & ATKINSON, R. (1973) An improved method for the isolation of fungi in onychomycosis. *British Journal of Dermatology*, **88**, 273.

ENGLISH, M.P. & ATKINSON, R. (1974) Onychomycosis in elderly chiropody patients. *British Journal of Dermatology*, **91**, 67.

ENGLISH, M.P., HARMAN, R.R.M. & TURVEY, J.W.J. (1967) *Pseudeurotium ovalis* in toenails. Some problems of mycological diagnosis of nail infections. *British Journal of Dermatology*, **79**, 553.

ENGLISH, M.P. & LEWIS, L. (1974) Ringworm in the South-West of England, 1960–1970, with special reference to onychomycosis. *British Journal of Dermatology*, **90**, 67.

GENTLES, J.C. (1971) Laboratory investigations of dermatophyte infections of nails. *Sabouraudia*, **9**, 149.

GENTLES, J.C. & EVANS, E.G.V. (1970) Infection of the feet and nails with *Hendersonula toruloidea*. *Sabouraudia*, **8**, 72.

JESSNER, M. (1922) Uber eine neue Form von Nagelmykosen (Leukonychia trichophytica). *Archive für Dermatologie und Syphilis (Berlin)*, **141**, 1.

KRASBACH, H. & HELIGE, H. (1964) Pathogene Pitzflora und Pilzkrankheiten im Einzugsgebeit der Grazer Hautklinik. *Archiv für Klinische und Experimentelle Dermatologie*, **220**, 60.

MARPLES, MARY J. (1965) *The Ecology of the Human Skin.* Charles C. Thomas, Springfield, Ill.

PUCCINELLI, V. & CECCARELLI, B. (1969) Contribution to the study of infection of the hair with tinea mycetes. *Archiv für Klinische and Experimentelle Dermatologie*, **234**, 250.

PUNITHALINGHAM, E. & ENGLISH, M.P. (1975) *Pyrenochaeta unguis-hominis* sp. nov. on human toe-nails. *Transactions of the British Mycological Society*, **64**, 539.

RAUBITSCHEK, F. (1961) Mechanical versus chemical keratolysis by dermatophytes. *Sabouraudia*, **1**, 87.

SOWINSKI, W. (1971) Trichophytic entrance sites into the nail in man. *Comptes rendus des Communications, V^{e} Congrès de la Société Internationale de Mycologie Humaine et Animale.* (Ed. by H. Drouhet) Institut Pasteur, Paris.

WALSHE, M.M. & ENGLISH, M.P. (1966) Fungi in nails. *British Journal of Dermatology*, **78**, 198.

WHITTLE, C.H. & GRESHAM, G.A. (1963) Paronychia experimentally induced. *Journal of Investigative Dermatology*, **40**, 267.

ZAIAS, N. (1972) Onychomycosis. *Archives of Dermatology*, **105**, 263.

ZAIAS, N., OERTEL, I. & ELLIOTT, D.F. (1969) Fungi in toe-nails. *Journal of Investigative Dermatology*, **53**, 140.

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RENAL FUNCTION IN METHOTREXATE TREATED PSORIATICS

SIR, Methotrexate (MTX) has an accepted place in the therapy of certain severe forms of psoriasis. Most of the complications seen with the higher dosages (upwards of 0·5 mg/kg/week) used to treat neoplastic diseases are rarely encountered with the schedules used for psoriasis (0·2–0·5 mg/kg/week). However, structural hepatic damage, occasionally progressing to cirrhosis, has been reported (Coe & Bull, 1968; Epstein & Croft, 1969; Weinstein *et al.*, 1970; Almeyda *et al.*, 1972), the earliest changes only being detectable by liver biopsy. Liver damage is seen much less when MTX is given orally once weekly (Dahl, Gregory & Scheuer, 1972; Warin *et al.*, 1975).

MTX is largely excreted by the kidneys (Henderson, Adamson & Olivero, 1965), but there are no comparable published studies of its effect on renal structure and function during MTX therapy of psoriasis.

Zachariae, Grunnet & Søgaard (1976) have presented renal biopsy data indicating that there are no structural changes in the renal parenchyma during MTX therapy. A prospective survey at The London Hospital suggests that there are no significant changes in renal function.

This report concerns twelve patients, all taking MTX orally once weekly. The age range for males is 25–67 years; for females 28–79 years. Serum creatinine, creatinine clearance and urinary albumin were measured before MTX therapy and at intervals thereafter. The results are summarized in Table 1.

Although there have been isolated reports of nephrotoxicity of MTX in psoriatics (Ryan & Vickers, 1966; McKenzie & Aitken, 1967; Ryan & Baker, 1969) renal damage attributable to the drug seems to be rare in psoriatics. In contrast, in a group of patients with advanced cancer and previously normal renal function, high doses of MTX (0·5–3·0 mg/kg) were reported to cause damage. The changes included a decrease in renal clearance of inulin and PAH, elevation of blood urea, and, in three cases who

TABLE 1. Serum creatinine and creatinine clearance data before and after MTX treatment of psoriasis

Case	MTX cumulative dose (mg)	Duration of treatment (months)	Serum creatinine† (mg/100ml)		24 h creatinine‡ clearance (ml/min)	
			Pre-MTX	Latest	Pre-MTX	Latest
1	1340	22	0·8	1·0	112	99
2	600	18	1·1	0·9	68	81
3	2300	34	1·1	1·1	68	80
4	2410	24	1·1	0·7	81	125
5	1600	30	1·1	1·0	135	138
6	540	40	0·9	1·0	94	105
7	910	13	0·5	0·6	110	115
8	1110	25	0·7	0·9	64*	92
9	1410	24	0·6	0·8	103	120
10	3100	48	0·7	0·7	77*	106
11	1580	38	0·8	0·9	66*	108
12	1985	42	0·8	1·0	120	95

* 3 h creatinine clearance.

† Normal value < 1·3 ml/100 ml.

‡ Normal value > 75 ml/100 ml.

No patient had albuminuria immediately before, or during MTX therapy of up to 4 years' duration.

died, there was extensive tubular damage (Condit, Chauls & Joel, 1969). However, the usual doses employed were far beyond the upper limit used in psoriasis.

Endogenous creatinine clearance and the detection of albuminuria are simple tests of glomerular and tubular function respectively. We have found abnormalities of neither, and there is no indication of deterioration of renal function from the data presented here. It is worth noting that impaired renal function from other causes is not a contra-indication to commencing MTX therapy where necessary, but calls for appropriate reduction in dose (Noble, White & Baker, 1975).

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REFERENCES

ALMEYDA, J., BARNADO, D., BAKER, H., LEVENE, G.M. & LANDELLS, J.W. (1972) Structural and functional abnormalities of the liver in psoriasis before and during methotrexate therapy. *British Journal of Dermatology*, **87**, 623.

COE, R.O. & BULL, F.E. (1968) Cirrhosis associated with methotrexate treatment of psoriasis. *Journal of the American Medical Association*, **206**, 1515.

CONDIT, P.T., CHAULS, R.E. & JOEL, W. (1969) Renal toxicity of methotrexate. *Cancer*, **23**, 126.

DAHL, M.G.C., GREGORY, M.M. & SCHEUER, P.J. (1972) Methotrexate hepatotoxicity in psoriasis—comparison of different dose regimens. *British Medical Journal*, **i**, 654.

EPSTEIN, E.H. & CROFT, J.D. (1969) Cirrhosis following methotrexate administration for psoriasis. *Archives of Dermatology*, **100**, 531.

HENDERSON, E.S., ADAMSON, R.H. & OLIVERO, V.T. (1965) The metabolic fate of tritiated MTX. II. Absorption and excretion in man. *Cancer Research*, **25**, 1018.

MCKENZIE, A.W. & AITKEN, C.V.E. (1967) Psoriasis and folic acid antagonists. *British Journal of Dermatology*, **79**, 122.

NOBLE, W.E., WHITE, P.M. & BAKER, H. (1975) Assay of therapeutic doses of MTX in body fluids of patients with psoriasis. *Journal of Investigative Dermatology*, **64**, 69.

RYAN, T.J. & VICKERS, H.R. (1966) Psoriasis and folic acid antagonists. *British Journal of Dermatology*, **78**, 612.

RYAN, T.J. & BAKER, H. (1969) Systemic corticosteroids and folic acid antagonists in the treatment of generalized pustular psoriasis. Evaluation and prognosis based on the study of 104 cases. *British Journal of Dermatology*, **81**, 134.

WARIN, A.P., LANDELLS, J.W., LEVENE, G.M. & BAKER, H. (1975) A prospective study of the effects of weekly oral methotrexate on liver biopsy. *British Journal of Dermatology*, **93**, 321.

WEINSTEIN, D.A., COX, J.W., SURINGA, D.W.R., MILLARD, M.M., KALSER, M. & FROST, P. (1970) Evaluation of possible chronic hepatotoxicity from methotrexate for psoriasis. *Archives of Dermatology*, **102**, 613.

ZACHARIAE, H., GRUNNET, E., & SØGAARD, H. (1976) Accidental kidney biopsy in psoriasis. *British Journal of Dermatology*, **94**, 655.

British Journal of Dermatology (1976) **94**, 704.

Book Review

Dermatitis Herpetiformis. J.O.'D.Alexander (1976) London: W.B.Saunders. Pp. 346, black/white illustrations.

Dr Alexander tells us in successive chapters about the history, clinical aspects, histopathology, ultrastructure, immunology, gastro-intestinal associations, genetics and treatment of dermatitis herpetiformis (DH). He also deals with pemphigus, pemphigoid and erythema multiforme alongside, and although one appreciates his reasons for doing this, it somewhat interrupts the 'flow' of the DH story.

One wonders, generally, about the usefulness of black and white photographs in dermatology but most of those in this book are excellent and convey the message intended for them. It would be unusual in a book with so many references not to find an occasional non-deliberate mistake (Pettit is spelt Petitt in the text but is correct in the references: was the important article in the alternate complement pathway by Provost & Tomasi (1973) really still 'In Press' in 1975 as stated ?).

The comprehensive collection of references is invaluable and is one good reason for buying the book. Another is the excellent section on histopathology which I'm sure many histopathologists as well as clinical dermatologists would benefit from reading.

The bulk of the text is factual reporting and where there are differences of opinion amongst different groups of workers in the DH field usually all sides of the argument are given. One or two very dogmatic statements which favour one view rather than the other presumably reflect the author's own opinion. It would be nice to know more of what he really thinks, for he has read all the references and should be in a better position than most to weigh the evidence and sum up.

The book illustrates well the various stages in the life history of DH, clinical description, histological differentiation, association with coeliac disease and immunological findings. Always, after the initial hope that each new stage held the key to understanding the disease, it later became apparent that it did not. Consequently, it is no criticism of the book to say that those who buy it in the hope of finding in it the ultimate answer to the riddle of DH, will be disappointed.

Janet M.Marks

British Journal of Dermatology (1976) **94**, 705.

Review

D-penicillamine in the treatment of rheumatoid arthritis and progressive systemic sclerosis

D-Penicillamine (B′B″ dimethylcysteine) is a drug widely known for its clinical therapeutic benefit in the treatment of Wilson's disease and cystinuria. A number of recent studies have demonstrated that penicillamine may be therapeutically active in other diseases including rheumatoid arthritis (RA), progressive systemic sclerosis (PSS), morphoea and active chronic hepatitis, as well as acting as a chelator of a number of heavy metals. The increasing number of therapeutic indications for D-penicillamine therapy need to be clearly defined and its ill effects plainly identified. This review will concentrate on the present value of this drug in the treatment of rheumatoid arthritis and progressive systemic sclerosis.

Pharmacological properties of D-*penicillamine* (*Fig.* 1)

Deutsch & Morton (1957) demonstrated that macromolecules, particularly macroglobulins, were dissociated by penicillamine *in vitro*. In view of this finding it appeared logical to prescribe penicillamine in the treatment of diseases in which macroglobulins play an important role, such as rheumatoid factor in rheumatoid arthritis and paraproteins in macroglobulinemia. In rheumatoid arthritis, penicillamine therapy is associated with a fall in rheumatoid factor titre and other immunoglobulin levels but it is not yet evident whether this is a primary effect or secondary to suppression of disease activity by another mechanism (Jaffe, 1968; Bluestone & Goldberg, 1973).

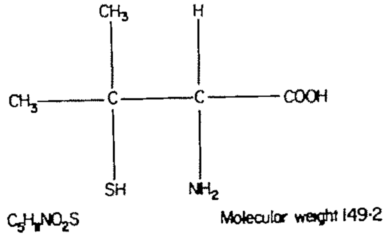

FIGURE 1. D-Penicillamine—B′B″ dimethylcysteine.

The lathyritic effects of D-penicillamine on collagen synthesis are well known and have formed the basis of the use of this drug in progressive systemic sclerosis (PSS) and morphoea. Nimni & Bavetta (1965) demonstrated that rats fed on penicillamine produced excessive amounts of collagen precursors, and subsequent studies have confirmed that this effect is a result of the inhibition of crosslinking of collagen. This may result in a delay in wound healing as demonstrated in experimental animals (Morris *et al.*, 1969). It has now been demonstrated that stability of the collagen molecule is dependent upon the formation of specific intermolecular crosslinks between tropocollagen molecules (Bailey, Peach & Fowler, 1970). In addition, immature or labile intermolecular crosslinks within the collagen molecule may be susceptible to cleavage by penicillamine (Fig. 2) (Bailey, 1968a). Such *in vitro* actions

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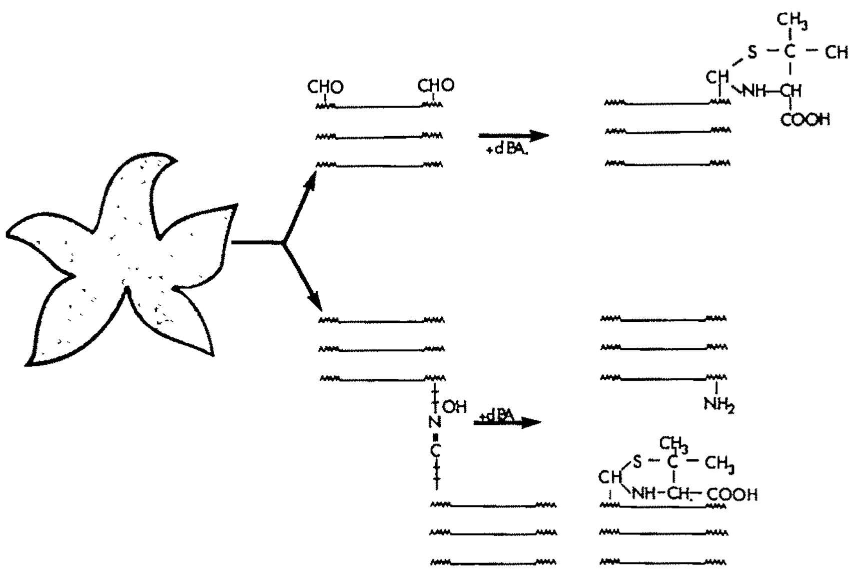

FIGURE 2. Effect of D-penicillamine on preventing inter-molecular cross linking of procollagen molecules (above) and the cleavage of labile aldimine crosslinks of collagen molecules (below).

may therefore have *in vivo* significance in diseases where disordered collagen metabolism is important, such as dermal collagen of PSS (Harris & Sjoerdsma, 1966) and possibly the proliferating synovium characteristic of RA.

The chelating effect of penicillamine on heavy metal ions has been the subject of several recent studies. It has been shown that patients with RA have high serum levels of copper associated with elevated caeruloplasmin levels (Lorber, Cutler & Chang, 1969). Similar changes have been demonstrated in other connective tissue diseases including PSS (Jayson *et al.*, 1976).

As copper is of importance in a number of co-enzyme systems, including some implicated in the biosynthesis of collagen, it is possible that rises in copper levels may be a non-specific reflection of connective tissue turnover and damage. Alternatively, chelation of copper by penicillamine might also result in an alteration of connective tissue synthesis.

Studies on the effect of penicillamine on the immune response have produced conflicting results. Delayed antibody response to injected antigen in experimental rabbits has been reported by several groups but this has been disputed by others. Furthermore, the effects of D-penicillamine on adjuvant arthritis in experimental animals have not been impressive (Liyanage & Currey, 1972). The fall in immunoglobulin levels in patients treated with penicillamine has, however, led Bluestone & Goldberg (1973) to suggest that penicillamine has an immunosuppressive action with selective suppression of immunoglobulin synthesis at a cellular level. One explanation for this is the demonstration that penicillamine will apparently inhibit *in vitro* lymphocyte transformation, by inhibition of the RNA dependent rather than the DNA synthetic phase of cellular division (Roath & Wills, 1974).

The property of D-penicillamine to inhibit viral replication is of speculative significance in the connective tissue diseases.

Penicillamine in the treatment of rheumatoid arthritis (RA)

The use of penicillamine in the treatment of RA has been controversial since the original report by Jaffe in 1964 that a patient with RA vasculitis and neuritis had responded to oral penicillamine therapy. It was not until the Multicentre Trial Group (MTG) report (1973) that some of the controversy surrounding this drug was allayed. In this controlled double-blind study, thirty and thirty-eight patients completed a 1-year trial of D-penicillamine and placebo, respectively. Using standard and widely accepted criteria for improvement of disease activity a significant improvement was noted in articular index, grip strength, functional assessment, ESR and haemoglobin in the penicillamine-treated group when compared with the controls. As patients were matched for age, sex and disease activity at the onset of the trial, the significance of the result was particularly impressive. In addition this study was heavily weighted against a favourable therapeutic response by the inclusion only of patients with severe disease which had not responded to orthodox treatment. Subsidiary problems have arisen from this report which have since become topics of further study. Firstly, penicillamine therapy is associated with a delay in a clinical response; and secondly it may cause a high incidence ($\simeq 30\%$) of potentially serious side effects.

Confirmatory studies of the results of the MTG have now appeared, although the drug has not been as widely studied in North America as in Europe. At the recent VIIIth European Rheumatology Congress in Helsinki, reports on the effectiveness of D-penicillamine in the treatment of RA were presented from Austria, Egypt, India, Russia, Germany, Yugoslavia and Spain. At the same congress, papers were presented which have thrown some light on the problems raised by the MTG report. Balme & Huskisson (1975) have reported that in their study on the chronopharmacology of penicillamine therapy in RA, there was often a delay in response to treatment which varied from 1 to 3 months but that the mean rate of improvement was greatest in the 2nd month. Two other studies showed that the dose of penicillamine used in the MTG study may not have been optimum. Hill & Hill (1975) reported that the withdrawal rate and incidence of side effects was greater in patients receiving up to 1500 mg when compared with those receiving 750 mg. Therapeutic efficacy did not appear to be lost although optimum maintenance dose did vary from patient to patient. In reporting the results of another multicentre study, Dixon *et al.* (1975) produced confirmatory evidence that the daily dosage should be kept as low as possible and that dosage increments should be made at infrequent intervals. Day and colleagues (1974) confirmed these conclusions. This 'go low, go slow' policy was not associated with any loss in therapeutic benefit, and met with widespread acceptance at the International workshop on penicillamine therapy held in London (October 1974).

In a recent multicentre control trial, D-penicillamine compared very favourably with gold therapy in the treatment of RA (Huskisson *et al.*, 1974). Further studies are indicated before the place of penicillamine will be finally decided.

Penicillamine in the treatment of progressive systemic sclerosis and morphoea

The underlying pathogenic mechanisms which lead to the development of both morphoea and progressive systemic sclerosis (PSS) have recently been clarified by the application of sophisticated biochemical analysis of the structural and functional variations in collagen. The stability of dermal collagen is dependent upon the inter-molecular crosslinking of tropocollagen molecules. These crosslinks are labile (soluble) aldimine bonds in newly formed collagen but become stable (insoluble) later (Bailey, 1968b). Herbert and her colleagues (1974) have clearly demonstrated that patients with active PSS have increased labile collagen crosslinks in skin biopsy specimens suggesting that a rapid proliferation of new collagen is occurring. The demonstrations by Harkness & Harkness (1966) that, *in vitro*, collagen is rendered fragile by D-penicillamine and by Bailey (1968a, b), that penicillamine cleaves labile crosslinks (see previous figure), have led to the use of penicillamine in the treatment of morphoea and PSS.

The assessments of therapeutic responsiveness *in vivo* of patients with PSS and morphoea have been limited by the lack of objective criteria of improvement. A number of early reports failed to show either subjective or objective evidence of improvement in PSS while others did report evidence of subjective improvement. In an attempt to obtain objective criteria some workers have used skin thickness and skin elasticity to assess reduction in skin collagen (Harvey, Henderson & Grahame, 1974). The value of these methods is disputed and personal use of them has failed to impress us.

In their original study Herbert and colleagues (1974) were able to demonstrate qualitative changes in skin collagen of patients with PSS when compared with normals. In some patients studied serially there was evidence that treatment with penicillamine led to a return of these qualitative changes to normal. In a review of these and other patients, biochemical evidence of qualitative improvement has been confirmed with varying subjective evidence of clinical improvement (Jayson & Davis, 1975). The major reservations with these and other studies are the absence of concurrent quantitative analysis, and inability to demonstrate to the satisfaction of critical reviewers that clinical improvement has occurred.

The present status of penicillamine therapy in PSS is confused by the fact that the natural history of the disease is not known. This drug is of probable benefit but there are reservations which will not be clarified until carefully controlled trials have been undertaken. There is, however, one salutary reminder as demonstrated in a recent patient seen personally (Davis, 1975). PSS is a systemic disease and in this case progression of systemic involvement occurred in a patient treated with penicillamine despite an unequivocal clinical and biochemical response by dermal collagen. As visceral collagen contains a different crosslink, which is not cleaved by penicillamine, it is possible that this apparent clinical paradox may reflect responsiveness of dermal collagen and not visceral collagen due to a fundamental difference in their crosslinking structure.

In morphoea, individual case reports have described remission and in some cases, complete resolution of skin lesions. Assessment of such reports is difficult to interpret, as morphoea is a disease in which spontaneous and unpredictable involution may occur. Moynahan (1974), however, has reported universal improvement in fourteen children with morphoea treated with penicillamine suggesting that in at least some patients therapy was beneficial.

Side effects of therapy

While D-penicillamine is of known benefit in RA and is probably beneficial in PSS and morphoea, its major limitation is toxicity. It is indeed unfortunate that the diversity of diseases for which penicillamine is prescribed is matched only by the diversity of side effects it produces.

Minor side effects are relatively common but often well tolerated and self-limiting. Fever, anorexia, nausea and vomiting may occur but in general patients can be 'nursed' through these early problems. In any assessment of side effects from a drug, it must be remembered that these are also associated with the use of a placebo and some of the minor reactions may not be related to the drug itself. Penicillamine, unlike many other drugs used in the treatment of RA, does not appear to alter back diffusion of hydrogen ions across the gastric mucosal barrier and is therefore unlikely to be ulcerogenic.

Hypogeusia has proved a consistent and sometimes poorly tolerated side effect. Henkin & Bradley have demonstrated that chelation of metal ions including copper, zinc and nickel will cause loss of taste and this observation was thought to explain hypogesia due to penicillamine chelation of metal ions. In initial studies addition of supplemental copper to penicillamine therapy was thought to prevent this problem (Henkin *et al.*, 1967), but more recent studies cast doubt on this observation (Lyle, 1974). Continuation or cessation of therapy usually results in a return of taste to normal without metal ion supplements, but permanent taste loss may occur rarely.

Skin reactions to penicillamine appear to take two basic forms. Early rashes which occur within the 1st month of treatment are usually maculopapular and pruritic, urticarial or eczematous (Scheinberg, 1968). These may necessitate the cessation of therapy although some physicians suppress the cutaneous reaction with steroids while continuing therapy. A later reaction usually occurring after the 6th month of treatment is similar in character to the rash seen in 'dry' beri-beri which has led to the speculation that it may be due to pyridoxine deficiency. A second later rash, elastosis perforans serpiginosa (Pass *et al.*, 1973), in which there may be bleeding into the skin over pressure points is also described. The exact nature of this lesion is not defined although it may be due indirectly to chelation of copper. Other dermatological reactions reported with penicillamine are dermatomyositis, mucous membrane ulceration, alopecia and hirsutism. Penicillamine may produce a drug-induced lupus-like illness (Walshe, 1968), and cross-reactivity in penicillin sensitive patients may be a rare cause of skin reactions (Assem & Vickers, 1974).

The potentially more serious side effects of penicillamine therapy are thrombocytopenia, leukopenia and nephropathy. A surprising and as yet unexplained fact is the higher incidence of these side effects in patients with RA and PSS when compared with patients receiving comparable doses of penicillamine for Wilson's disease and cystinuria.

Thrombocytopenia is a relatively common side effect. In the MTG report (1973) a fall in platelet count was common after starting therapy although this fall was only severe enough to warrant stopping therapy in two patients. Although falling platelet counts may occur at any time during the course of therapy it is usually an early and gradual fall. Precipitate falls are uncommon and therefore routine platelet counts are a worthwhile procedure for monitoring and preventing this side effect, which is reversible if therapy is stopped. Neutropenia and aplastic anaemia are less predictable than thrombocytopenia but both are fortunately rare.

Penicillamine nephropathy has been recognized for many years in patients with cystinuria but is surprisingly infrequently seen in Wilson's disease. Nephropathy has also been frequently reported in patients receiving penicillamine for RA and PSS. This side effect first presents with proteinuria which may be minimal and transient or progressive, and lead to a frank nephrotic syndrome. Proteinuria may in some cases be associated with haematuria. Most clinicians regard persistent proteinuria of greater than 1G per 24 h and/or haematuria as positive indications for stopping therapy. Proteinuria of <1G per 24 h is probably in the view of most physicians not an indication for cessation, although our personal practice and that of others is to stop therapy if proteinuria exceeds 200 mg/24 h. After the drug is discontinued proteinuria usually resolves spontaneously although not always as rapidly as early reports would suggest. Persistence of proteinuria over 1 year may occur, although there is little evidence to date to suggest that chronic renal impairment will result (Huskisson & Hart, 1972). The nature of the renal damage with penicillamine has led to some interesting studies. Routine light microscopy of renal biopsies usually reveals little change, although immunofluorescent studies have demonstrated deposition of immunoglobulin and C3 suggesting the presence of immune complexes. Electronmicrography (EM) has confirmed this with the demonstration of electron dense deposits adjacent to the basement membrane. These changes, however, do not appear to be associated with changes in circulating immune complexes or complement consumption as is seen in the nephropathy of other immune complex diseases such as systemic lupus erythematosus (Bacon, Tribe & Mackenzie, 1975). The hapten responsible for nephropathy has as yet been unidentified but there is some evidence to suggest that it is not penicillamine. An alternative theory (Verrier-Jones, 1975) is that renal damage by penicillamine releases a renal tubular antigen which may be the hapten of the immune complex, in a similar way to gold nephropathy. Further experience on the natural history of penicillamine nephropathy will be gained with longer follow-up periods and repeat biopsy programmes.

A wide number of other side-effects have been reported to penicillamine therapy, but in most cases the association is not clearly defined. One side effect which has been recently documented although of unknown pathogenesis is a myasthenia gravis-like reaction (Bucknall *et al.*, 1975; Balint *et al.*, 1975).

Similarly, cases of pemphigus have occurred among patients with rheumatoid arthritis treated with D-penicillamine (Hewitt, Benveniste & Lessana-Leibowitch, 1975).

Synopsis

D-Penicillamine is a therapeutically active drug in rheumatoid arthritis and may also prove effective in progressive systemic sclerosis. Its major limiting factor is toxicity and as such the use of D-penicillamine should be limited to those who are experienced and willing to monitor its use. Toxicity can be kept to a minimum by using the lowest therapeutically active dose and by checking patients at regular intervals for thrombocytopenia and proteinuria. These and other side effects are usually reversible if diagnosed early.

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REFERENCES

ASSEM E.S.K. & VICKERS, M.R. (1974) Immunological response to penicillamine in penicillin-allergic patients and in normal subjects. *Postgraduate Medical Journal* (Suppl. 2), **50**, 65.

BACON, P.A., TRIBE, C.R. & MACKENZIE, J.C. (1975) Penicillamine induced nephropathy in rheumatoid arthritis. *Scandinavian Journal of Rheumatology* (Suppl. 8), **4**, Abstr. 21–10.

BAILEY, A.J. (1968a) Intermediate labile intermolecular crosslinks in collagen fibres. *Biochimica et biophysica acta*, **160**, 447.

BAILEY, A.J. (1968b) The nature of collagen. *Comprehensive Biochemistry*, Vol. 263 (Ed. by M.Glorkin and E.H.Stotz), p. 247. Elsevier, Amsterdam.

BAILEY, A.J., PEACH, C.M. & FOWLER, L.J. (1970) Chemistry of the collagen crosslinks. *Biochemical Journal*, **117**, 819.

BÁLINT, G., SZOBOR, A., TEMESVÁRI, P., ZAHUMENSZKY, Z. & BOZSOKY, S. (1975) Myasthenia gravis developed under d-penicillamine treatment. *Scandinavian Journal of Rheumatology* (Suppl 8), **4**, Abstr. 21–12.

BALME, H.W. & HUSKISSON, E.C. (1975) Chronopharmacology of penicillamine and gold. *Scandinavian Journal of Rheumatology* (Suppl. 8), **4**, Abstr. 21–07.

BLUESTONE, R. & GOLDBERG, L.S. (1973) Effect of d-penicillamine on serum immunoglobulins and rheumatoid factor. *Annals of Rheumatic Diseases*, **32**, 50.

BUCKNALL, R.C., DIXON, A.SR.J., GLICK, E.N., WOODLAND, J. & ZUTSHI, D.W. (1975) Myasthenia gravis associated with D-penicillamine treatment for rheumatoid arthritis. *British Medical Journal*, **i**, 600.

DAVIS, P. (1975) Renal involvement in progressive systemic sclerosis. *Proceedings of the Royal Society of Medicine*, **68**, 544.

DAY, A.T., GOLDING, J.R., LEE, P.N. & BUTTERWORTH, D. (1974) Penicillamine in rheumatoid disease: A long-term study. *British Medical Journal*, **i**, 180.

DEUTSCH, H.F. & MORTON, J.I. (1957) Dissociation of human serum macroglobulins. *Science*, **125**, 600.

DIXON, A.SR.J., DAVIES, J., DORMANDY, T.L., HAMILTON, E.B.D., HOLT, P.J.L., MASON, R.M., ZUTSHI, D.W., THOMPSON, M. & WEBER, J.C.P. (1975) Synthetic penicillamine in rheumatoid arthritis. Comparison of high and low dose regimens. A double-blind controlled trial. *Scandinavian Journal of Rheumatology* (Suppl. 8), **4**, Abstr. 21–09.

HARKNESS, M.L.R. & HARKNESS, R.D. (1966) Effects of sulphydryl reagents on a mechanical property of skin. *Nature (London)*, **211**, 496.

HARRIS, E.J. & SJOERDSMA, A. (1966) Effect of penicillamine on human collagen and its possible application to treatment of scleroderma. *Lancet*, **ii**, 996.

HARVEY, W., HENDERSON, D.R.F. & GRAHAME, R. (1974) Observations on *in vivo* skin elasticity and skin thickness of patients with rheumatoid arthritis treated with D-penicillamine and gold salts. *Postgraduate Medical Journal* (Suppl. 2), **50**, 33.

HENKIN, R.I. & BRADLEY, D.F.R. (1969) Regulation of taste acuity by thiols and metal ions. *Proceedings of U.S. National Academy of Science*, **62**, 30.

HENKIN, R.I., KEISER, H.R., JAFFE, I.A., STERNLIEB, I. & SCHEINBERG, I.H. (1967) Decreased taste sensitivity after D-penicillamine reversed by copper administration. *Lancet*, **ii**, 1268.

HERBERT, C.M., BAILEY, A.J., JAYSON, M.I.V. & LINDBERG, K.A. (1974) Scleroderma: Biosynthesis and maturation of skin collagen and the effects of D-penicillamine. *Lancet*, **i**, 187.

HEWITT, J., BENVENISTE, M. & LESSANA-LEIBOWITCH, M. (1975) Pemphigus induced by D-penicillamine: an 'experimental' pemphigus. *British Journal of Dermatology* (Suppl. 11), **93**, 12.

HILL, H.F.H. & HILL, A.G.S. (1975) Penicillamine in rheumatoid arthritis. *Scandinavian Journal of Rheumatology* (Suppl. 8), **4**, Abstr. 21–05.

HUSKISSON, E.C., GIBSON, T.J., BALME, H.W., BERRY, H., BURRY, H.C., GRAHAME, R., DUDLEY HART, F., HENDERSON, D.R.F. & WOJTULEWSKI, J.A. (1974) Trial comparing D-penicillamine and gold in rheumatoid arthritis. Preliminary report. *Annals of the Rheumatic Diseases*, **33**, 532.

HUSKISSON, E.C. & HART, F.D. (1972) Penicillamine in the treatment of rheumatoid arthritis. *Annals of the Rheumatic Diseases*, **31**, 402.

JAFFE, I.A. (1964) Rheumatoid arthritis with arteritis. Report of a case treated with penicillamine. *Annals of Internal Medicine*, **61**, 556.

JAFFE, I.A. (1968) Penicillamine in rheumatoid disease with particular reference to rheumatoid factor. *Postgraduate Medical Journal* (Suppl.), **44**, 34.

JAYSON, M.I.V. & DAVIS, P. (1975) Penicillamine therapy in the treatment of scleroderma: a study of its effects on collagen. *Scandinavian Journal of Rheumatology* (Suppl. 8), **4**, Abstr. 21–17.

JAYSON, M.I.V., WHICHER, J., DAVIS, P. & WALTERS, G. (1976) Studies of copper metabolism in ankylosing spondylitis, systemic sclerosis and morphoea. *Annals of Rheumatic Diseases* (in press).

LIYANGE, S.P. & CURREY, H.L.F. (1972) Failure of oral D-penicillamine to modify adjuvant arthritis or immune response in the rat. *Annals of Rheumatic Diseases*, **31**, 521.

LORBER, A., CUTLER, L.S. & CHANG, C.C. (1968) Serum copper levels in rheumatoid arthritis: relationship of elevated copper to protein alterations. *Arthritis and Rheumatism*, **11**, 65.

LYLE, W.H. (1974) Penicillamine and zinc. *Lancet*, **ii**, 1140.

MORRIS, J.J., SEIFTER, E., RETTURA, G., GEEVER, E.F. & LEVENSON, S.M. (1969) Effect of penicillamine upon healing wounds. *Journal of Surgical Research*, **9**, 143.

MOYNAHAN, E.J. (1974) Penicillamine in the treatment of morphoea and keloid in children. *Postgraduate Medical Journal* (Suppl. 2), **50**, 39.

MULTICENTRE TRIAL GROUP (1973) Controlled trial of D-penicillamine in severe rheumatoid arthritis. *Lancet*, **i**, 275.

NIMNI, M.E. & BAVETTA, L.A. (1965) Collagen defect induced by penicillamine. *Science*, **150**, 905.

PASS, F., GOLDFISCHER, S.G., STERNLIEB, I. & SCHEINBERG, I.H. (1973) Elastosis perforans serpiginosa during penicillamine therapy for Wilson's disease. *Archives of Dermatology*, **108**, 713.

ROATH, S. & WILLS, R. (1974) The effects of penicillamine on lymphocytes in culture. *Postgraduate Medical Journal* (Suppl. 2), **50**, 56.

SCHEINBERG, I.H. (1968) Toxicity of penicillamine. *Postgraduate Medical Journal* (Suppl.), **44**, 11.

VERRIER-JONES, J. (1975) Personal communication.

WALSHE, J.M. (1968) Toxic reaction to penicillamine in patients with Wilson's disease. *Postgraduate Medical Journal* (Suppl.), **44**, 6.

British Journal of Dermatology (1976) **94**, 712.

News and Notices

The Egyptian Society of Dermatology and Venereology in association with The Pan Arab and Middle East Dermatological Association

An International conference will be held in Cairo from 20 to 24 February 1977 (President: Prof. M.El Zawahry). A Symposium on 'Psoralens in Dermatology' (Chairman: Prof. A.M.El Mofty) will be held on 24 February 1977.

Dermatologists from different parts of the world are invited to participate. A session on 'Venereology' and 'Andrology' will be included. The official languages will be English, French, German and Arabic. Applications should be submitted before 31 October 1976. Application forms may be obtained from the Secretary General: Prof. H.El Hefnawi, Dar El Hekma, 42 Kasr El Aini St., Cairo, Egypt.

International Symposium on Psoriasis

The Second International Symposium on Psoriasis will be held at Stanford University from 12 to 15 July 1976; cosponsored by the National Institute of Arthritis, Metabolism, and Digestive Diseases. The topics to be covered will include newer developments in basic biology, chemistry, immunology, and genetics relating to psoriasis; natural history and epidemiology of psoriasis; skin structure and function in psoriasis; therapy for psoriasis—day care centers, climatotheraphy, topical and systemic chemotherapy, photochemotherapy. The tuition fee is $200 ($100 for residents in training). For further information please write to Paul H. Jacobs M.D., Department of Dermatology, Stanford University School of Medicine, Stanford, California 94305, U.S.A. Telephone (415) 497-6101.

International Society of Tropical Dermatology Symposium on Climate and Ecology in Skin Diseases, Las Palmas, Canary Islands 1–3 November 1976

This Symposium is another regional program of International Society of Tropical Dermatology. The first of these programs was in Guadalajara, Mexico in December 1975, and the second in New Delhi, India in February 1976. This particular program will be Chaired by Dr Arthur Rook, President of the International Society of Tropical Dermatology and the program has been organized by Dr Alejandro de Gyorko-Gyorkos of Las Palmas, Canary Islands; Dr Roger Harman of Bristol, England; Dr Jose Mascaro of Valencia, Spain; and Dr Sigfrid Muller of Rochester, Minnesota,

Anyone wishing to attend, or submit titles for free communications, should contact Dr Alejandro de Gyorko-Gyorkos, Villa-Hungria, Las Perreras 63, Las Palmas, Canary Islands.

Erratum

In the Book Review of *Haut und Anhangsgebilde: Spezielle Histopathologie* (Band 7 of Spezielle Pathologische Anatomie) edited by U.W. Schnyder, which appeared in the January issue of this Journal, the year of publication should read **1973**.

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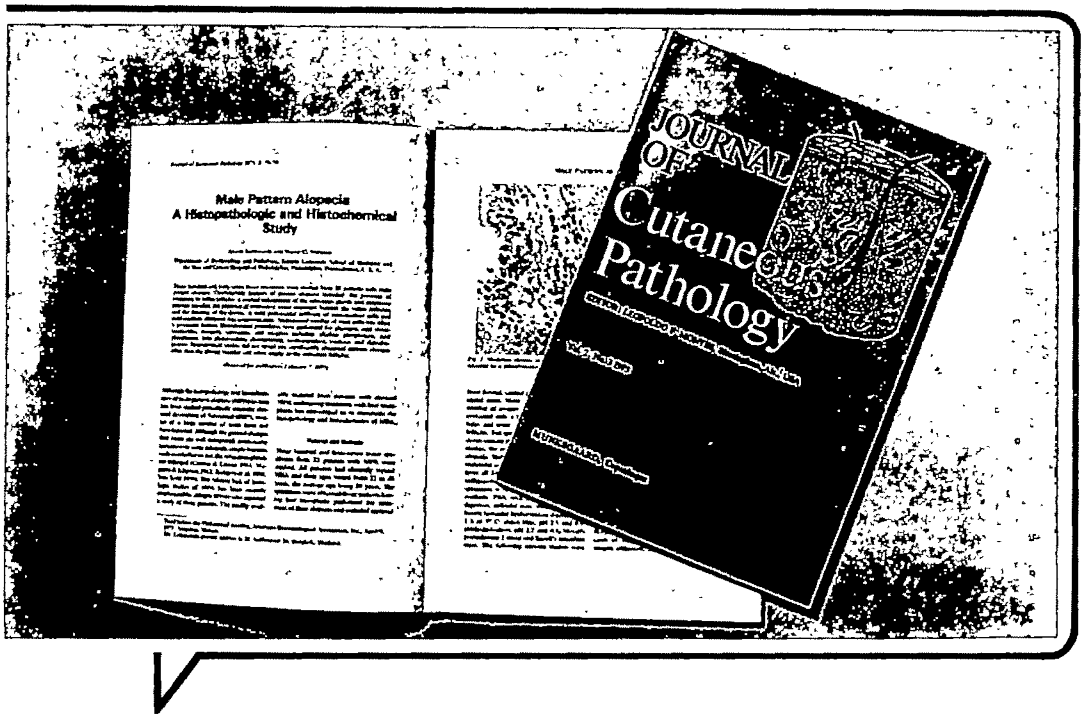
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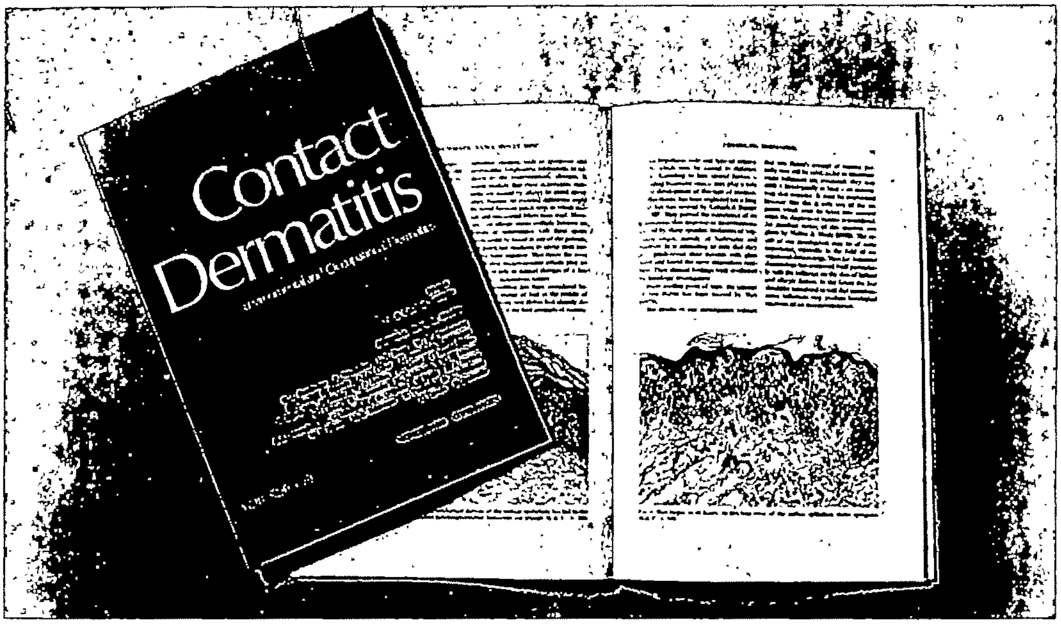

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kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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Toxic Woods

by Brian Woods and
C. D. Calnan

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Toxic Woods

BRIAN WOODS* AND C.D.CALNAN†

SUMMARY

Eighty-three cases of dermatitis caused by wood or sawdust have been seen at St John's Hospital for Diseases of the Skin, London, in the last 20 years. The clinical details and results of patch tests are reported. The literature on dermatitis, mucosal irritation, asthma and other toxic effects of woods of some 300 botanical species is reviewed at length, with an index of scientific, trade and vernacular names, chemical formulae of the known sensitizing substances, and a table of the 28 toxic woods most often encountered. Pathogenesis, diagnosis and prophylaxis are discussed.

KEY WORDS

Contact dermatitis
Industrial dermatitis
Urticaria
Asthma
Hay fever
Wood
Liverworts
Lichens

INTRODUCTION

The baleful effects of some trees have always been a favourite subject for mythology, and in early would-be scientific texts like Pliny's *Natural History* the borderline between fact and fiction was sometimes hazy. His striking account of travellers in Gaul poisoned by wine from a flask of yew wood did not convince Evelyn (1664), who was more inclined to blame the wine than the wood. 'How are poor Trees traduc'd'. Gerard (1597) quoted his own experience to refute the persistent notion that the very shade of the yew tree was harmful.

One might be equally sceptical about the belated story (Richard, 1843) of the French soldiers in Napoleonic times who were taken ill after eating meat cooked on spits of oleander wood, if there were not modern reports of similar incidents in America (Hausen, 1970). As early as the seventh century dermatitis due to Japanese lacquer was known in the Orient, and it spread to Europe with the eighteenth-century vogue for chinoiserie (Bateman, 1819). The properties of other tropical trees with caustic sap, such as the manchineel and rengas, were wildly exaggerated in travellers' tales reaching Europe, culminating in the curious myth of the deadly Upas tree (Woods, 1962).

The occupational risks from sawdust with which this paper is mainly concerned were first clearly described by Ramazzini (1700), who referred to irritation of the nose and eyes, most marked in pit-sawyers. He named no woods as particularly troublesome except cypress, the smell of which gave some turners a headache. For two centuries little or nothing was added to this by medical writers. In 1902 Oliver published *Dangerous Trades* which included an account of coryzal symptoms in men

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working with sequoia wood, and in the same year Young described reactions to a boxwood substitute (see para. *156*). In the next few years teak, rosewood and satinwood dermatitis were reported, but most publicity went to 'African boxwood' (*183*), which caused such alarming symptoms in Lancashire shuttlemakers that men refused to work with it and a full clinical and biochemical investigation had to be undertaken (Legge, 1905, 1907).

Poisoning by colonial timbers may have been regarded with some schadenfreude at first in Germany as a subject in which England had a right to lead the field, but similar reports soon came from German authors. A review by Czimatis & Hagemann (1910) was widely read, and their list of toxic woods copied by other writers together with their botanical names. Unfortunately these were not always accurate, especially with American species. Many timbers were (and are) known in the trade by vague generic terms such as rosewood or satinwood. Identification of the trees from which they come is not always easy even for expert botanists, of whom there were very few in tropical America 70 years ago. By the time Record & Hess published their *Timbers of the New World* in 1943 most of the difficulties had been cleared up (largely in Record's department at Yale), but some mythical toxic woods still live on from earlier days in much-copied lists (see *Ferolia 55*, *Rhodorhiza 186*, and probably *Coccoloba 38*).

France's principal contribution was to show that European timbers like oak and chestnut could also cause dermatitis, though more in forest-workers than carpenters and joiners. It was quickly found that the bark contained the bulk of the sensitizer, which was eventually traced to liverworts and lichens growing on the trees and undergrowth (Le Coulant & Lopès, 1956). The sensitizer has been identified as a sesquiterpene lactone (*24*). Most of the allergens in major commercial timbers have now been isolated and their structure determined, notably by workers in Hamburg in the last 15 years (Schulz & Dietrichs, 1962).

CLINICAL EFFECTS

Toxic woods, nearly always in the form of sawdust, may affect the skin or mucous membranes by direct contact or may be absorbed and produce general symptoms. The skin can be damaged by the mechanical action of bristles and splinters, by chemical irritation or sensitization, or by a combination of these. Photosensitization has not been proved but may well occur in the Rutaceae and Flindersiaceae.

Irritant dermatitis

Irritant chemicals are often found in the sap or latex, and are characteristic of certain families such as the Moraceae, Urticaceae, Euphorbiaceae and Apocynaceae. Some trees contain irritant powders in the bark or in cracks in the wood (brigalow *60*, tagayasan *72*, araroba *97*). Both types of irritant, being confined largely to the outer part of the tree, produce their worst effects when it is felled, and are well known to local timber-workers. Sometimes the bark is burnt off before the tree is felled, or the trunk may be left for some time to dry before being sawn up. Very strong sensitizers, like those of the Anacardiaceae, affect so many workers that they are treated as irritants, though it is often known locally that some men are immune. Teak and some other woods are both irritants and sensitizers, even after seasoning, and this makes patch tests hard to interpret.

The clinical picture is that of any irritant dermatitis, with erythema and blistering of the exposed skin, often complicated by erosions and secondary infection in the poor hygienic conditions under which many lumbermen work and live (Freise, 1932–37; Suskind, 1967). The conjunctivae are often severely affected (manchineel *123*, geor *125*). Mild irritants may affect only susceptible areas such as the fingerwebs and corners of the mouth (Hornsey, 1914).

Sensitization dermatitis

Sensitizing chemicals are more often found among the extractives of the heartwood and therefore affect sawyers, carpenters and joiners, polishers and finishers exposed to fine dust. The picture is that of an airborne contact dermatitis, beginning on the dorsa of the hands and forearms, eyelids, face and neck, and rapidly involving the genitals through handling in micturition. In bald men the scalp is often affected. Longin (1929) emphasized the *triade éruptive* of hands, face and genitals in forest workers sensitive to liverworts on bark. If the clothing gives poor protection fine dust may drift inside it and affect the sweaty areas of the axillae, waistband and groins, and sometimes the ankles and dorsa of the feet.

Mild cases have only erythema and slight irritation, but often there is papular or vesicular dermatitis which may progress to chronic dermatitis after repeated exposure. Severe fissured lichenification of the palms was described as *mains de crocodile* by Morand (1907).

The latent period varies from a week to many years, but is most commonly 10–15 days (Touraine & Golé, 1934). In successive attacks it becomes shorter and the eczema usually increases in severity, but hardening is not unusual (Schwartz, 1931). Cross-reactions to other woods, or other sources of related antigens, are common (Merrill, 1944).

Dermatitis from the solid wood of finished articles is much less common, but has followed prolonged contact with musical instruments, bracelets or knife handles made of the strongly allergenic *Dalbergia* rosewoods (*94*). Furniture made of rengas (*129*) or obeche (*146*) can also give trouble.

Other skin lesions

Bristles or hairs from the bark, leaves or other parts of the tree sometimes irritate the skin (bamboo *18*, palm *19*, elm *25*, ramin *157*, dogwood *166*) or mucous membranes (plane *54*) of lumbermen and foresters. Even caterpillar hairs may be responsible (pine *7*, acacia *60*). Splinter wounds from a number of woods (Douglas fir *5*, greenheart *44*, acacia *60*, afrormosia *83*, limba *159*, lancewood *163*) are slow to heal and often become septic. It would be interesting to look in these for the granuloma formation characteristic of wounds from blackthorn spines (Buhr, 1960) and some cactus spines (Schreiber *et al.*, 1971). Folliculitis was described by Freise (1936) with several woods in Brazil, and by Salamone *et al.* (1969) with mansonia (*147*). Tagayasan (*72*) and araroba (*97*) sawdust stain the skin violet or brown, and white peroba (*191*) yellow. Tonka (*99*) and lapacho woods (*190*) can cause striking colour changes in the hair.

Oehling (1963) described contact urticaria as characteristic of obeche (*146*) sensitivity, but urticarial reactions have also been reported with larch (*9*) and iroko (*32*). Oehling was able to reproduce the urticaria within 5 min by rubbing the patient's skin with a slip of obeche but not with other woods. Many of the woodworkers he tested gave positive intradermal tests with obeche extract, even among those who did not get urticaria or asthma on exposure to the wood at work. Several substances have now been reported to cause contact urticaria (Maibach & Johnson, 1975), probably not always through an immune mechanism (Calnan & Shuster, 1963).

Mucosal irritation

Nasal irritation and sneezing are particularly common with certain woods (sequoia *10*, western red cedar *13*, sabicu *57*, dahoma *62*, sneezewood *109*, guarea *117*, mansonia *147*, makoré *170*, opepe *195*), some of which have little or no effect on the skin. Dryness and soreness of the throat may extend to the larynx and trachea. Epistaxis and even haemoptysis may be conspicuous (stavewood *119*, opepe *195*) and headache is common. Nausea may lead to vomiting and anorexia lasting several days (stavewood *119*, Knysna boxwood *183*). Conjunctivitis usually accompanies nasal irritation but it is also characteristic of some skin reactions (lichens *24*, araroba *97*).

Irritation of the upper respiratory tract is generally found in most of the workers exposed, but asthma affects only a minority. It has been described with sawdust of western red cedar (*13*), iroko (*32*), amboyna (*93*), box (*127*), obeche (*146*), teak (*188*), and less often with a number of other woods. Attacks of asthma may be delayed for several hours after exposure to the sawdust, which sometimes obscures the diagnosis (Gandevia & Milne, 1970). Inhalation tests are more reliable than scratch or intradermal tests, which give both false positives and false negatives (Sosman *et al.*, 1969). Desensitization by injections of kejaat extract (*93*) was successful in the hands of Ordman (1949b), but has proved disappointing with most woods.

Gade (1921) maintained that carpenters often suffered from asthma due to beech sawdust and had a high mortality from respiratory disease. This was denied by Vallander (1950), who found only one case of asthma and one with radiographic pulmonary infiltration among ninety men with at least 10 years' experience in woodworking. There are a few woods, however, which cause recurrent bronchitis or pneumonitis leading to chronic lung damage (sequoia *10*, cork oak *24*, maple *141*).

Acheson *et al.* (1968) found a much increased incidence of adenocarcinoma of the paranasal sinuses in English woodworkers and there are similar reports from France (Fombeur, 1972). Black *et al.* (1974) tested nasal mucociliary activity in woodworkers and found considerable impairment, especially in men with over 10 years' exposure to dust. No particular wood or constituent has been incriminated, but Schoental (1973) showed that some unsaturated carbonyl compounds in lignins were carcinogenic for rats. The evidence for a connection between woodworking and Hodgkin's disease in the U.S.A. is less convincing (Milham & Hesser, 1967; Acheson, 1967; Spiers, 1969). Pine pollen is not now thought to be a significant cause of sarcoidosis (Scadding, 1967), though it is allergenic and can produce sarcoid-like granulomas in experimental animals.

Systemic symptoms

Headache, salivation, thirst and nausea are sometimes too severe to be explained by simple mucosal irritation (red peroba *179*, Knysna boxwood *183*). Giddiness, drowsiness, visual disturbances, colic, muscle cramps and cardiac arrhythmia suggest the effects of alkaloids or glycosides absorbed through the respiratory or alimentary tracts, or occasionally through skin abrasions. Pharmacologically active substances have been demonstrated in the bark or wood of many trees known to have such effects (barberry *39*, missanda *65*, ferro *71*, sophora *85*, laburnum *88*, wenge *90*, Knysna boxwood *183*, opepe *195*). Dixon (1911) thought the amounts absorbed would be so small that there must be an 'idiosyncrasy' rather than simple pharmacological action, but the incidence of these effects is generally too high for an allergic mechanism.

In the days when food was eaten from wooden containers and implements it was common knowledge that certain woods did not taint the food (see spindle-tree *140*, dogwood *166*). Others were avoided because they contained strongly flavoured extractives such as turpentine (Boulger, 1906). In exceptional cases poisoning was recorded from containers, spoons or spits made of woods like yew (*2*) or oleander (*184*) which harbour toxic alkaloids.

WOOD CHEMISTRY

The structural components of wood are cellulose, hemicellulose and lignin, but it is the accessory substances or 'extractives' found mainly in the heartwood that are responsible for most toxic effects. Vorreiter (1949/1958) classifies these as follows: (1) fats, resins, oils and waxes; (2) proteins, gums, latex, mucus, starch, sugars; (3) alkaloids, bitter principles, dyes, tannins, glycosides, camphor, perfumes, etc.; (4) inorganic and organic acids and salts; (5) minerals.

Some of these act as food reserves for latent growth periods, some as hardening agents, and others protect against mechanical injuries or attack by bacteria, fungi, insects and larger animals (Dietrichs, 1958). Some are metabolic by-products or end-products of no apparent use to the tree.

TOXIC SUBSTANCES

Alkaloids and **glycosides** were among the first substances isolated and studied by plant chemists, though establishment of their complex structures often had to wait till recent times. Those of no medical or economic importance are still largely undetermined. Systemic effects, pharmacological rather than allergic, are characteristic of these substances, which have been found in many toxic woods and barks (pigeon plum *38*, greenheart *44*, mesquite *61*, missanda *65*, ferro *71*, basralocus *74*, wenge *90*, guatambu *104*, Ceylon satinwood *108*, avodiré *118*, red peroba *179*, Knysna boxwood *183*, opepe *195*, abura *196*) as well as those shown in Fig. 1. The structure of the taxines (from yew *2*) is complex (Merck, 1968).

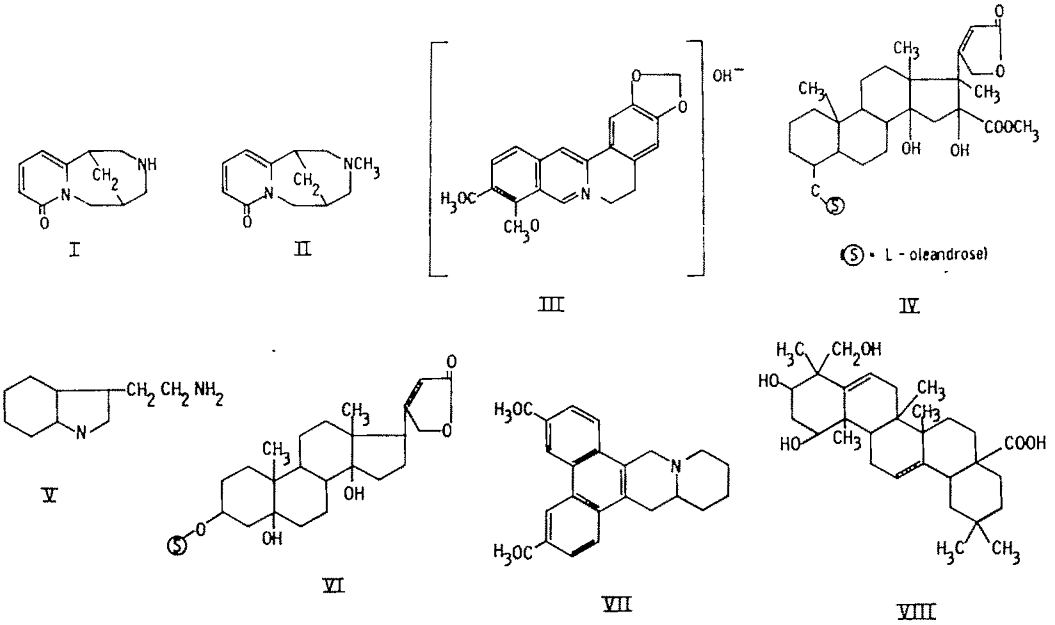

FIGURE 1. Alkaloids, glycosides, saponins. I. Cytisine; II. *N*-Methyl cytisine; III. Berberine; IV. Oleandrin; V. Tryptamine; VI. Periplocin; VII. Cryptopleurine; VIII. Bassic acid (aglycone of makoré-saponin).

Saponins are found in large quantities in the wood of satine (*29*) and in smaller amounts in many others (albizia *56*, vinhatico *64*, black bean *81*, mahua *167*, makoré *170*, etc.). The aglycone of makoré saponin is shown in Fig. 1. Saponins are haemolytic when given parenterally and are sometimes used in arrow poisons. They irritate mucous membranes but do not penetrate unbroken skin.

Many of the strongest skin-sensitizers are found among the **phenols** (Fig. 2), especially the **catechols** of the poison-ivy family (*128*). The only other families in which similar phenols are commonly found are the Proteaceae (*35*) and Ginkgoaceae (*17*), which are surprisingly distant taxonomically from the Anacardiaceae.

Some active **quinones** (Fig. 3) from woods have been known since the nineteenth century. Lapacho is found mainly in the Bignoniaceae (*190*, *191*) but also in teak (*188*) and some other woods. Chrysophanic acid used to be extracted from *Vataireopsis* (*97*) in the form of chrysarobin for treating psoria-

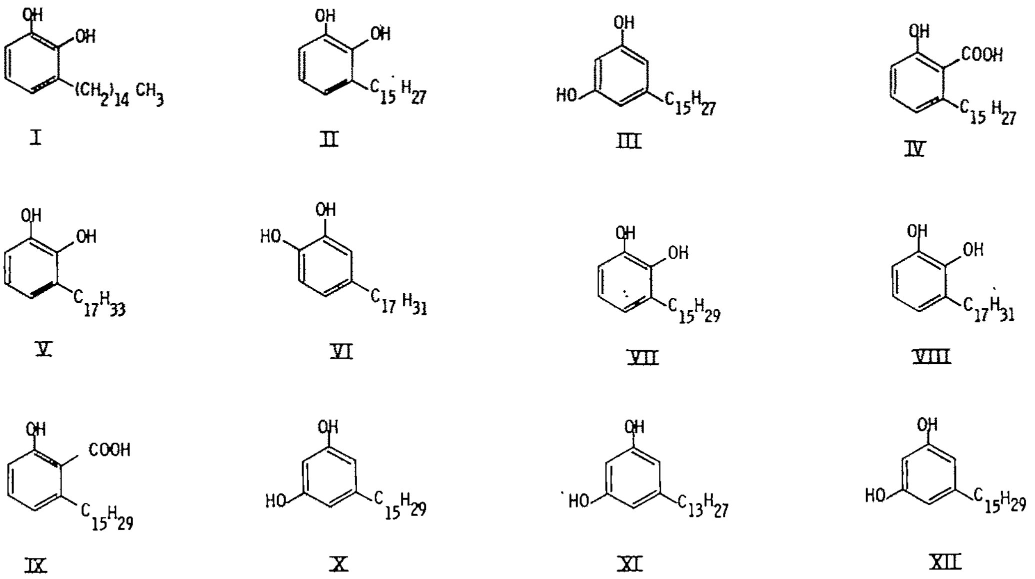

FIGURE 2. Phenols. I. 3-Pentadecylcatechol (hydrourushiol); II. Urushiol (8,11,14 tri-enyl in *Rhus toxicodendron*, 8,11,13 tri-enyl in *R. vernicifera*); III. Cardol; IV. Anacardic acid; V. Glutarenghol; VI. Thitsiol; VII. Renghol; VIII. Laccol; IX. Ginkgolic acid; X. Bilobol; XI. Grevillol; XII. 5-(10-Pentadecenyl)resorcinol.

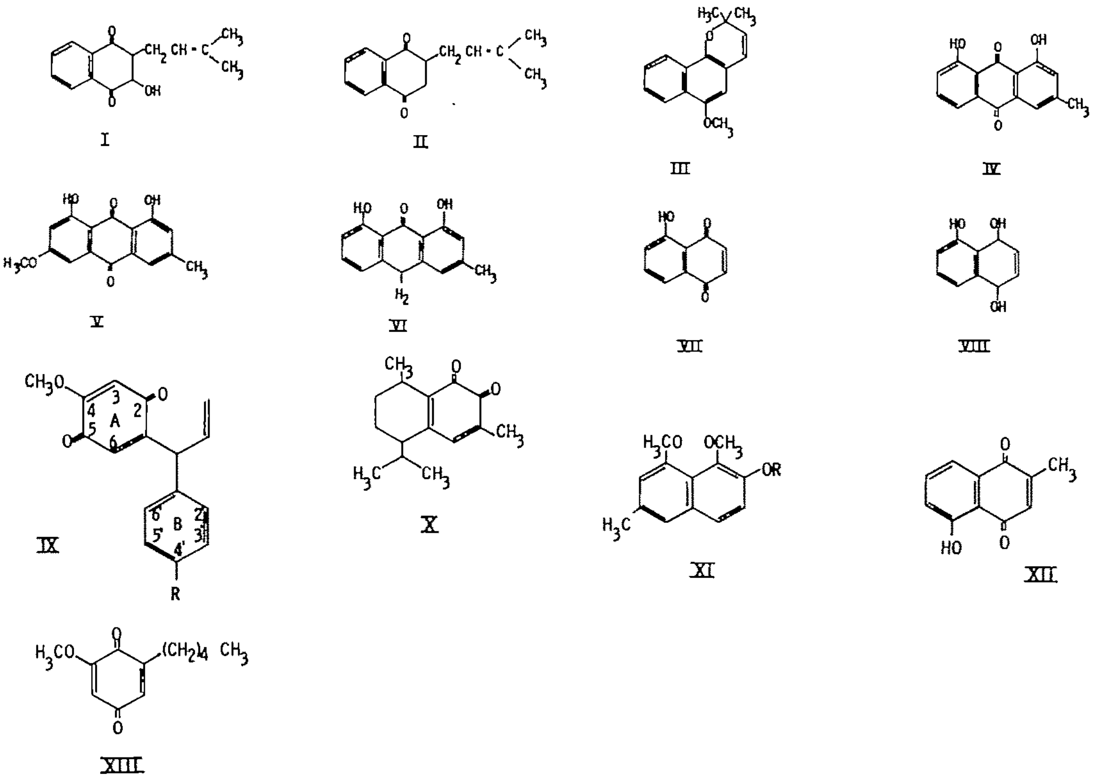

FIGURE 3. Quinones and related substances. I. Lapachol; II. Desoxylapachol; III. Lapachenol (lapachonone); IV. Chrysophanic acid; V. Physcion; VI. Chrysophanic acid anthrone; VII. Juglone; VIII. α-Hydrojuglone; IX. Dalbergiones (4-methoxydalbergione: R = H; 4,4′-dimethoxydalbergione: R = OCH_3; 4′-hydroxy 4-methoxydalbergione: R = OH); X. Mansonone-A; XI. Makassars (makassar II: R = H; makassar III: R = CH_3); XII. Plumbagin; XIII. Primin.

sis, and it is also found in *Vatairea* (*98*) and tagayasan (*72*). In both cases the active agents are not the quinones themselves, but the less stable related substances, desoxylapachone and chrysophanic acid anthrone. Juglone, or its precursor α-hydrojuglone, is found in the sensitizing shells of walnuts (*21*) but not in the wood. Plumbagin is a strongly irritant quinone found in the bark of some *Diospyros* spp. (*173*).

Recently other groups of allergenic quinones have been demonstrated in mansonia (*147*) and the *Dalbergia* rosewoods (*94*), the dalbergiones in particular being very strong sensitizers which have also been found in some other woods (purpleheart *68*, angelin *96*). The macassars found in ebony heartwood (*173*) can easily be oxidized to quinones, but these are not yet known to sensitize (Hausen, 1970). Hausen and other German workers have demonstrated but not yet identified possible skin-sensitizing quinones in other woods (American whitewood *41*, sucupira *82*, cocus *91*, Jamaica quassia *110*, *Cordia* spp. *187*).

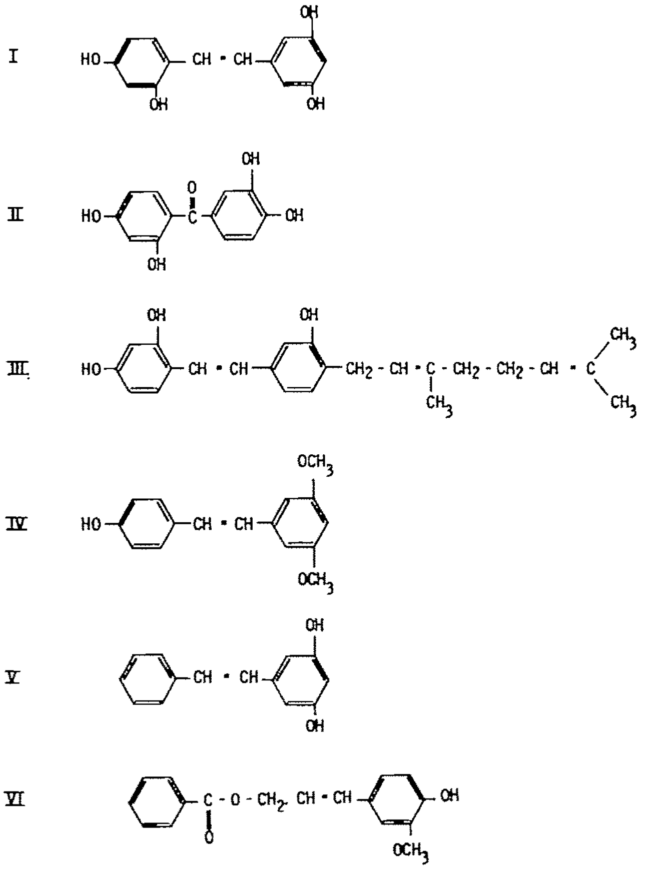

FIGURE 4. Stilbenes. I. 2,3',4,5' tetrahydroxystilbene; II. Maclurin; III. Chlorophorin; IV. Pterostilbene; V. Pinosylvine; VI. Coniferyl benzoate.

Primin, the sensitizer in *Primula obconica*, was shown by Schildknecht *et al.* (1967) to be a quinone not unlike the *Dalbergia* quinones (Fig. 3), and cross-reactions between them have been observed by Hjorth *et al.* (1969).

A number of **stilbenes** (Fig. 4) occur in allergenic woods, but only chlorophorin (iroko *32*) and coniferyl benzoate (Peru balsam *84*) are known to sensitize. **Terpenes** (Fig. 5) include delta-3-carene from turpentine (pine *7*, etc.), sesquiterpene lactones in *Frullania* and other sensitizing liverworts found on bark (*24*), and euphorbol and other complex terpenes of uncertain toxicity found in the latex of Euphorbiaceae (*122*).

The photosensitizing **furocoumarins** (Fig. 6) are widespread in the Rutaceae and Flindersiaceae and may be at least partly responsible for skin reactions to woods of these families (West Indian, African and Ceylon satinwoods *103*, *108*), but this has not yet been proved. Bergaptene (8-methyl psoralen), the cause of berloque dermatitis, was first obtained from the peel of the Bergamot orange (*Citrus aurantium* var. *bergamia; 106*). The latex of fig trees also contains a photosensitizing furocoumarin (Kuske, 1939).

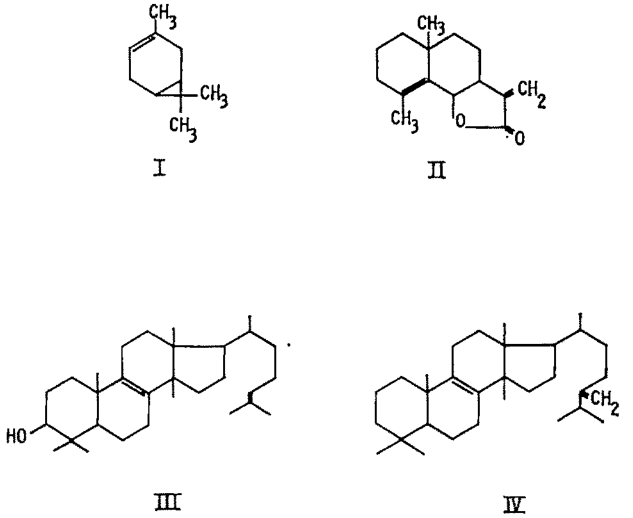

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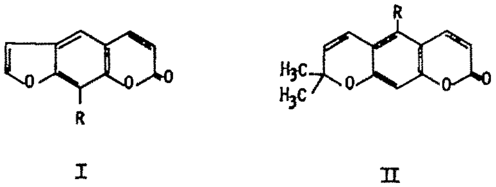
FIGURE 5. Terpenes. I. Δ-3 Carene; II. Eudesmanolide sesquiterpene lactone; III. Euphol; IV. Euphorbol.



I II

FIGURE 6. Coumarins. I. Psoralen (R = H); 8-Methylpsoralen (R = CH_3); xanthotoxin (R = OCH_3); II. Xanthyletin (R = H); xanthoxyletin (R = OCH_3).

The formulae of some other known or suspected sensitizers are shown in Fig. 7.

Reference has been made to sensitizing substances penetrating into wood from liverworts and lichens on the bark. Many other chemicals are introduced into timber while it is being processed. Preservatives such as pentachlorophenol and other chlorinated phenols (Schwarzkpof, 1954; Fournier, 1965; Crow, 1970); potassium dichromate (Grossmann, 1920; Behrbohm, 1957); urea-formaldehyde- and epoxy- resins, turpentine, polishes, varnishes and dyes (Meneghini & Rubini, 1958; Hering, 1966) cause much more dermatitis in woodworkers than the woods themselves (Suskind, 1967; Tan & Mitchell, 1968).

DIAGNOSIS

The first step is to establish what timbers and additives the patient has been exposed to. The full trade name of each timber is necessary, together with the country of origin. Whenever possible wood samples (not sawdust) should be sent to experts for identification; the staff of Forest Products Research Laboratories, Institutes of Forestry and Botanical Gardens are often helpful beyond the call of duty.

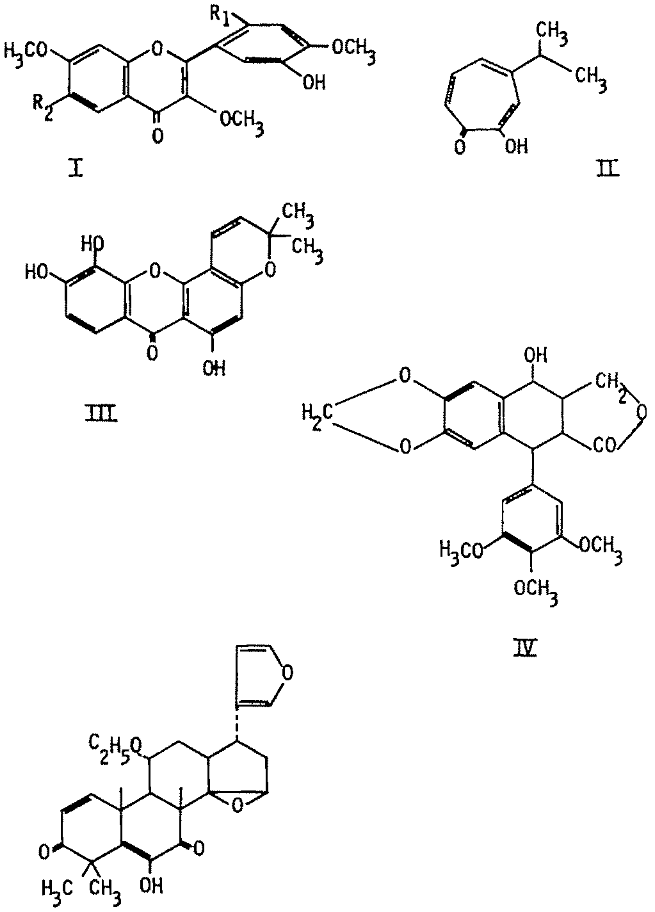


FIGURE 7. Other substances. I. Ayanin (R_1 = OH, R_2 = H); oxyayanin-A (R_1 = OH, R_2 = H); oxyayanin-B (R_1 = H, R_2 = OH); II. β-thujaplicin (tropolone); III. Jacareubin (xanthone); IV. Podophyllotoxin (picropodophyllin is a stereo-isomer); V. Anthothecol.

With known irritants such as mansonia (*147*) further investigation is unnecessary if the symptoms produced are typical. Where skin sensitization is suspected, however, patch tests should be made with fine sawdust (to avoid mechanical irritation). Moistening of the sawdust is advisable except with teak where it leads to false positive results. Positive tests with woods not known to be sensitizers should be checked by several tests on control subjects. Patch testing with the pure sensitizing substances, where known, is not yet practicable in routine diagnosis.

Bronchial sensitivity is best examined by inhalation tests with aqueous extracts of the sawdust, adequately controlled. Prick tests and intradermal tests are unreliable and difficult to interpret. Circulating antibodies have been demonstrated in some cases (sequoia).

PROPHYLAXIS

Compared with other trades, woodworking does not carry a high incidence of industrial dermatitis (Zschunke, 1963), and most of the trouble that does occur is due to additives rather than the wood itself. Although the numbers are small, however, the circumstances may be dramatic when a traditional wood is replaced by a substitute and many workers are smitten at once by an unfamiliar toxic substance. Complete prevention of such outbreaks is hardly possible, with the constantly shifting pattern of timber imports and the difficulty of identifying logs and especially sawn timber. The manufacturer and even the shipper may be unaware of a few toxic logs among harmless ones (see *Machaerium 95*, *Vatairea 98*).

Some timbers like teak, mansonia and rosewoods have such valuable properties that they are still used despite their known toxicity. The main preventive measures with such woods are dust control, protective clothing, hygiene, barrier creams and personnel selection.

(1) *Dust control.* Hanslian & Kadlec (1964a) showed that mechanical polishing produced on average 40 mg (maximum 200 mg) per cubic metre of wood dust in the surrounding air, and that 90% of the particles were less than 5 μm in diameter. They recommended that the maximum permitted level should be 1 mg/m^3 for 'toxic' woods such as yew, box and mansonia; 5 mg/m^3 for moderately active woods like pine, larch, ebony and mahogany; and 10 mg/m^3 for other woods. The American Conference of Governmental Industrial Hygienists recommended in 1970 that not more than 5 mg/m^3 should be allowed, and this was the average found in High Wycombe factories by Hounam & Williams (1974), though there were some much higher levels. Baumer (1955) describes and illustrates methods of exhaust ventilation suitable for vertical moulders, polishers, circular saws and other machines. There is good evidence of the efficiency of exhaust ventilation in general (Krogh, 1964), but it is less effective with certain processes (Legge, 1907) and with very fine dusts. Moistening the air is sometimes helpful (Komatsu, 1963).

(2) *Protective clothing* includes a high collar, long sleeves and when practicable gauntlets for skin-irritants, and masks for mucosal irritants. It tends to be neglected in hot conditions when increased sweating also makes men more vulnerable.

(3) *Washing* facilities should include showers where there is a risk of dust getting under the clothes.

(4) *Barrier creams* are more popular with lawyers than doctors. Even when they are not irritating in themselves, manual work quickly removes them from the exposed areas of skin which are most at risk.

(5) *Nasal sprays* with antihistamines were found useful against the very irritating dust of *Thuja standishii* (*13*) by Komatsu (1963), but they would hardly be suitable for prolonged use.

(6) *Personnel selection* is to some extent automatic as men who know they are sensitive to certain woods can usually avoid them, management being co-operative in a craft industry. Prospective patch-testing before employment is unreliable, and there is a risk that the test itself may sensitize the man.

PREVIOUS REVIEWS OF TOXIC WOODS

Reviews written before about 1930 are apt to be unreliable both botanically and chemically. Touton's (1932) exhaustive but not always critical monograph on plant dermatitis in Jadassohn's Handbuch is still useful for older references. Senear (1933) and Rasch (1935) wrote reviews in English which are now of mainly historical interest. Local studies exist for Australia (Cleland, 1914, 1925, 1932), Brazil (Freise, 1932, 1936, 1937), Malaya (Ridley, 1922; Burkill, 1935), Panama (Allen, 1943) and India (Behl & Captain, 1966).

Sandermann & Barghoorn's (1956) well-documented review has been superseded by Hausen (1970), who is particularly strong on wood chemistry. Unfortunately Hausen's comprehensive thesis is difficult to obtain, but he has published an abridged version without references (Hausen, 1973).

There are also a number of reviews which consist of little more than lists of supposedly toxic woods, without adequate documentation. Many woods have found their way into such lists on quite insufficient evidence or through mistaken identity. Some of them have been included in the present review simply to demonstrate the lack of evidence against them. Where there are convincing clinical details and/or patch tests, they have always been mentioned here. Known chemical constituents of each wood are generally given only if there is reason to think they are toxic.

Willis (see below) has been followed for botanical nomenclature and also for the order of families (Engler system) and genera. Closely related trees should therefore usually be found close together here, though there is of course still much disagreement among taxonomists, especially over the larger groups. British Standard names of timbers are printed in **bold**, and international *noms pilotes* from the Nomenclature Générale shown by (NP). (See below).

BOTANICAL AUTHORITIES

British Standard Nomenclature of Commercial Timbers. B.S. 881 and 589 (1955) in: Commonwealth Forestry Handbook (1962), Empire Forestry Association, London.

Nomenclature Générale des Bois Tropicaux (1965) Association technique internationale des bois tropicaux, Nogent-sur-Marne.

A Dictionary of the Flowering Plants and Ferns (1966) WILLIS, J.C., rev. AIRY SHAW, H.K. 7th edn. Cambridge University Press.

Handbook of Hardwoods (1972) rev. FARMER, R.H. 2nd edn. Ministry of Technology, HMSO, London.

Handbook of Softwoods (1957) Ministry of Technology, HMSO, London.

World Timbers (1969) RENDLE, B.J. Benn, Toronto and London.

The Structure of Wood (1970) JANE, F.W. 2nd edn, rev. WILSON, K. & WHITE, D.J.B. Black, London.

GYMNOSPERMAE: CONIFERAE Softwoods

PODOCARPACEAE

1. *Dacrydium cupressinum* Soland.	Rimu

The sawdust of this New Zealand wood causes temporary spasmodic rhinorrhoea in some workers (Cleland, 1925).

TAXACEAE

2. *Taxus baccata* L.	Yew

A keen archer had severe dermatitis on the face, neck, hands and forearms each time he scraped his bow (Senear, 1933). He patch-tested himself with moist sawdust from the bow and from the block of English yew it was made of, with positive results. Grossmann (1920) had experienced mild headache and discomfort while working with yew, as had other workers with him. Moll (1950) says that it irritates the respiratory passages as well as the skin, but quotes no cases.

Most parts of the tree, including the wood, contain the series of alkaloids called taxines which are responsible for yew's internal toxicity (Merck, 1968; Hausen, 1970), but there is also an irritant volatile oil (Forsyth, 1968).

Pliny's story of travellers in Gaul poisoned by wine from hip-flasks made of yew-wood is always quoted, but there is little from more reliable sources. Poisoning of men and cattle by yew leaves is of course well known.

ARAUCARIACEAE

3. *Araucaria angustifolia* O. Ktze. (*A. brasiliana* A. Rich.)	**Parana pine,** pin parana (NP), araucaria

A trade report of fever, weakness and muscle pains after working with Parana pine was not investigated clinically, but the wood was found to contain at least two alkaloids (Hausen, 1970). One St John's patient gave a positive patch test with Parana pine (*211*).

PINACEAE

4. *Picea sitchensis* Carr.	**Sitka spruce,** silver spruce
P. abies Karst. (*P. excelsa* Link.)	**European spruce,** whitewood

Women cutting up and sandpapering silver spruce in an aircraft factory developed irritating vesicles on the hands (Prosser White, 1920). Fregert & Rorsman (1963) saw a case of contact sensitivity to the balsams of pine and spruce, and found many unexplained positive results when these were included in routine patch tests. Cross-reactions with stilboestrol suggested that hydroxystilbenes might be responsible (but see *7*). Bahn (1928) obtained strongly positive intradermal tests with spruce and pine wood extracts in a sawmill worker with asthma; oak and beech gave weaker reactions and control subjects were negative. Cobe (1930) had two asthmatic patients who became sensitive to the needles of *P. mariana* B.S.P. (black spruce), with positive intradermal tests.

One St John's patient gave positive patch tests with spruce and with casein glue (*215*).

5. *Pseudotsuga menziesii* (Mirb.) Franco (*P. taxifolia* Britt.)	**Douglas fir,** Oregon pine, British Columbia pine

A messenger at the Forest Products Research Laboratory with slight exposure to many woods developed severe dermatitis on exposed skin. Patch tests were strongly positive with Douglas fir and negative with several other woods (Wilkinson, 1971). Suskind (1967) patch-tested eighty-two British Columbia lumber workers with eczema of the hands, and found two reacted to an extract of Douglas fir wood and one to grand fir (*6*). There are many trade complaints of septic splinter wounds (Nordin, 1947; FPRL, 1963). Neudörffer's (1902) case of general symptoms after wreath-making with Douglas fir is not convincing.

The wood contains α-pinene and the resin thunbergol and Δ-3-carene (Hancock & Swan, 1965).

6. *Abies alba* Mill. (*A. pectinata* DC.)	**Silver fir,** whitewood
A. grandis Lindl.	**Grand fir,** white fir, western balsam
A. balsamea (L.) Mill.	**Balsam fir**

Ganzoni (1929) described eczema of the hands and arms spreading to the face and neck, which recurred each winter in farmers who felled and sawed silver fir at that season only. Patch tests with fir resin were strongly positive. One of Suskind's lumbermen (see *5*) gave positive patch tests with the bark and leaves of grand fir, but not with the wood. Some firs have sensitizing lichens or liverworts (see *24*) growing on their bark (Tenchio, 1948). Kappes (1948) described contact dermatitis (with positive patch test) in a florist, from the foliage of balsam fir (the tree which produces Canada balsam). Siemens (1929) reported dermatitis from the foliage of silver fir, but made no patch tests. Twelve cases of contact dermatitis from 'fir' wood were seen at the Finsen Institute between 1935 and 1963 (Hjorth, 1965).

7. *Pinus sylvestris* L.	**Scots pine,** redwood, red deal
P. pinaster Ait.	**Maritime pine,** cluster pine
P. palustris Mill.	**Pitch pine**

Peri (1910) described irritation of the hands by shavings of maritime pine used in working cast iron. Wilkinson (1971) has seen five cases of contact dermatitis in carpenters, who had positive patch tests with sawdust and extracts of pine wood. It is not known whether the sensitizer is a stilbene such as pinosylvine (King, 1953) or a balsam element like Δ-3-carene (Pirilä *et al.*, 1964) or coniferylbenzoate (Figs 4 and 5).

Woodcutter's eczema (*24*) occurs in pinewoods, either from lichens on conifer bark or liverworts on undergrowth (Le Coulant *et al.*, 1966). Katzenellenbogen (1955) described dermatitis in Israeli foresters working on pines infested with the caterpillar *Thaumatopoea pinivora*. Patch tests were positive with caterpillar hairs (?irritation) but negative with the pulped cocoon. Allergy to pine pollen is not now considered a significant aetiological factor in sarcoidosis (Scadding, 1967).

There is no evidence that pitch pine is toxic. Hausen (1970) showed that it had been included in some lists through a blunder over the name.

Six St John's patients gave positive patch tests with pine or 'deal' (*210*).

8. *Cedrus deodara* (Roxb. ex Lamb.) G.Don — Deodar
   (*C. libani*, *Pinus deodara*)

The oil extracted by distillation of the wood is used as an insect repellent and antiseptic (Gamble, 1902), but the wood, sap and cones can irritate the skin (Behl & Captain, 1966).

9. *Larix decidua* Mill. — **European larch**
   (*L. europea* DC.)

Hanslian & Kadlec (1964) described contact urticaria from larch sawdust with positive patch tests. Chromatography showed Δ-3-carene and other terpenes in the wood (Fig. 5). Schulmann & Détouillon (1932) and Tenchio (1948) included larch among the trees that cause woodcutter's eczema (*24*).

TAXODIACEAE

10. *Sequoia sempervirens* Endl. — **Sequoia,** Californian redwood

Oliver (1902) described coryzal symptoms in men sawing and chipping sequoia wood. They complained of running at the nose, sneezing, coughing, laboured breathing, oppression at the pit of the stomach and smarting in the eyes, and were noted to have tachycardia. The symptoms were worst on the first exposure and lasted about 24 h, but tolerance later developed except in asthma subjects. Oliver put the sawdust into animals' cages; rabbits were unaffected, but some rats ran at the nose.

MacPherson (1925b) reported similar symptoms in Australian cabinet-makers. Cohen *et al.* (1967) described a case of chronic granulomatous pneumonitis (sequoiosis) with circulating antibodies to redwood sawdust (or possibly to an associated fungus) in a sawmill worker.

CUPRESSACEAE

11. *Callitris* spp. — Cypress pine

Cleland (1925) quoted a very brief reference to dermatitis caused by cypress pine cut when in flower. *C. articulata* contains thymoquinone (see *12*).

12. *Calocedrus decurrens* (Torr.) Florin — **Incense cedar** (California)
   (*Libocedrus decurrens* Torr.)

Calnan (1972) reported two cases of pencil dermatitis, one on the hand in a clerk and the other behind the ear in a carpenter. Patch tests with shavings from the pencils were positive, and one patient was also positive to extracts of incense cedar and western red cedar (*13*). Both reacted strongly to 1% thymoquinone and hydrothymoquinone, but a primary irritant effect was not ruled out. Neither reacted to β-thujaplicin.

Incense cedar heartwood contains carvacrol, p-methoxythymol, p-methoxycarvacrol, terpenes, α-thujaplicin and small amounts of β thujaplicin and β-thujaplicinol as well as thymoquinone and hydrothymoquinone (Anderson & Zavarin, 1955). Incense cedar and Virginian pencil cedar are officially-recognized causes of dermatitis in the German pencil industry (Koelsch, 1931).

13. *Thuja plicata* D.Don — **Western red cedar,** beisugi, giant arbor vitae

T. standishii Carr. — Arbor vitae, nezuko

T. standishii is an important timber in Japan, where its fine dust causes rhinitis in almost 90% of workers and asthma in a smaller number (Komatsu, 1963; Ito, 1963). Ito found *T. plicata* rather less troublesome to woodworkers, but there were a number of cases of 'beisugi asthma' among people living in houses rebuilt with it after the 1923 Tokyo earthquake (Hausen, 1970). Doig (1949) described asthma from *T. plicata* sawdust in England, and Gandevia & Milne (1970) reported in detail six cases of asthma and four of rhinitis in Australian woodworkers. The asthma often came on at night several hours after exposure to the dust, and would persist for some days after avoiding it (Mitchell, 1970). Skin tests were unreliable, but bronchial inhalation tests were positive, and the F.E.V. was slightly reduced on working days even in men without clinical asthma (Gandevia, 1970).

Bleumink & Nater (1972) described a case of dermatitis in a sawyer who gave strongly positive patch tests with western red cedar and a weak positive with deal. γ-Thujaplicin gave a strongly positive reaction, and another tropolone a delayed positive. The FPRL (1963) receives many trade complaints of skin as well as mucosal irritation, but no other case reports of dermatitis have been found.

Bridge (1937) described severe nasal irritation produced in one man, and possibly bronchitis in two others, by the sawdust of 'Canadian red cedar'. This is more likely to have been *T. plicata* than *Juniperus virginiana* (eastern red cedar).

Ito (1964) sensitized guinea-pigs with β-thujaplicin, but it is still not clear whether this is the cause of asthma in humans (Chan-Yeung *et al.*, 1971).

Thuja occidentalis L. — **White cedar,** eastern arbor vitae

Sachs (1926) described dermatitis of the hands affecting several men working with white cedar; another man had pharyngitis and bronchitis but no dermatitis.

14. *Cupressus sempervirens* L. — Cypress

Ramazzini (1700) remarked that some turners suffered from headaches due to the strong smell of cypress wood. Lewith (1929) reported transient erythema from a patch test with cypress sawdust in a man sensitive to palisander. No other cases have been found, but the wood is now rarely used. It is known to contain beta-thujaplicin and several terpenes (Enzell & Erdtman, 1957).

15. *Chamaecyparis lawsoniana* Parl. (*Cupressus lawsoniana* A.Murr.) — **Port Orford cedar,** Lawson's cypress

Asthma due to cedar wood has been described on the west coast of the U.S.A. (Schonwald, 1929; Stier, 1929). Prosser White (1929) found splinter wounds from it were slow to heal. The powerful resinous odour of the newly-cut wood was said to cause troublesome diuresis in saw-mill workers (Sargent, 1896).

None of Hausen's references for contact dermatitis from the wood is convincing on scrutiny. Unlike most Cupressaceae, Lawson's cypress contains very little beta-thujaplicin and no other tropolones (Zavarin *et al.*, 1959).

16. *Juniperus sabina* L.	Savin, savinier (NP), sabinenwacholder
J. oxycedrus L.	Cade
J. virginiana L.	**Virginian pencil cedar,** eastern red cedar

Moll (1950) seems to follow Grossmann (1920) in listing savin as toxic, but no reports of ill-effects from this little-used wood have been found. Oil of cade from the berries of *J. oxycedrus* is irritant, and also sensitizes some patients (White, 1887; Gougerot & Blamoutier, 1922a). The leaves of most junipers are irritant (White, 1889) and the pollen of *J. mexicana* Spreng. is a common cause of hay fever in Texas (Wodehouse, 1971).

J. virginiana is an officially recognized cause of dermatitis in Germany (see *12*). Cedarwood oil from this species has been suspected of phototoxicity (Opdyke, 1974).

GINKGOALES GINKGOACEAE

17. *Ginkgo biloba* L. Maidenhair tree

The pulp of the berries can cause contact dermatitis (Bolus, 1939). The ginkgolic acid and bilobol responsible (Fig. 2) are similar to the sensitizing catechols in *Rhus* spp. and may cross-react with them (Sowers *et al.*, 1965). Dermatitis from the little-used timber has not been reported.

ANGIOSPERMAE Hardwoods

GRAMINEAE

18. *Bambusa vulgaris* Schrad. et spp. (and other genera) Bamboo

The bristles on the leaf and stem sheaths are known to be irritating (Ridley, 1924; Allen, 1943), but Schiff (1951) reported dermatitis in workers sanding bamboo stems from which the bark and hairs had been removed. Patch tests with bamboo shavings were positive, but no control subjects were tested.

PALMAE

19. *Astrocaryum* spp., *Bactris* spp., etc. Palms

Freise (1937) described dermatitis of the arms and general symptoms in men working with palm wood in Brazil. Patch tests with a wax extracted from the wood were positive. The leaf-spines and fruit irritate the skin (Allen, 1943).

Cocus or cocos wood (*91*) has nothing to do with the coconut palm (*Cocos nucifera* L.).

SALICACEAE

20. *Populus albus* L.	**White poplar,** abele
P. carolensis Foug.	Carolina poplar, carolin
P. gileadensis Rouleau	Balm of Gilead

Winkler (1956) described two cases of dermatitis in woodmen who had been in contact with resin from white poplar buds. Patch tests with the resin and with turpentine were positive on both men and negative on two controls. A less convincing case of dermatitis from the sawdust of an unspecified American poplar was reported by Weber (1953). Poplar is one of the trees listed by Schulmann & Détouillon (1932) as causes of woodcutter's eczema (*24*). According to Le Coulant *et al.* (1966) enough allergen soaks into the wood from liverworts on the bark to sensitize workers making match splints from 'carolins d'Italie' (*P. carolensis* or a hybrid?) in southern France.

Dermatitis due to balm of Gilead was well described by White (1887), when it was a common domestic salve obtained from *P. gileadensis* in the U.S.A.

JUGLANDACEAE

21. *Juglans regia* L.	**European walnut**
J. nigra L.	**American walnut,** black walnut

The nutshells of both species stain the skin dark brown and can cause dermatitis. A German report (Barniske, 1957) suggested sensitization, but an American one (Siegel, 1954) favoured a primary irritant effect. The active substance is probably the xanthoquinone juglone (Fig. 3), a precursor of which is found in the nutshells, roots and bark, but not in the wood (Brissemoret & Combes, 1905; Hausen, 1970). No case reports of skin or mucosal irritation by the wood have been found. One man at St John's gave a positive patch test with *J. regia* veneer, but he reacted to so many woods and other allergens that the significance of the tests was doubtful (*218*).

BETULACEAE

22. *Alnus glutinosa* Gaertn.	**Common alder,** black alder

Brügel & Perutz (1927) described dermatitis of the face and hands in workers using black alder from Russia. Patch tests with the wood, sawdust, aqueous and alcoholic extracts and alder tannin were positive in all four and negative in controls. Cross-reactions with catechu, rheum and hamamelis suggested that the sensitizer was a catechol in the tannin. Dässler & Urzynicok (1958) extracted and purified the yellow crystalline tannin from black alder bark, and obtained negative patch tests on forty-one people. Only two of these had had alder dermatitis, however, and it was not stated how long ago.

23. *Betula papyracea* Ait.	**Paper birch,** white birch,
(*B. papyrifera* Marsh.)	American birch (in part)

Senear (1933) quoted a report in a trade journal that workmen in mills where paper birch logs were used often had a skin eruption due to the fine powder on the bark.

FAGACEAE

24. *Fagus sylvatica* L. **Beech**
Quercus robur L. et spp. **Oak**
Castanea sativa Mill. **Sweet chestnut**

Horand (1907) gave the name 'mains de crocodile' to chronic fissured hyperkeratotic eczema of the hands in woodmen working with chestnut, which he attributed mainly to tannin. Spillmann (1921) described dermatitis on the ear and side of the neck in a man who had carried freshly-cut oak logs on his shoulder, and suggested that fungi on the bark might be responsible. Tommasi (1929) reported similar cases from Italy and suspected an 'idiosyncrasy' because an attack often followed returning to work with wood after an interval. Dubreuilh (1931) described six cases of 'woodcutter's eczema' and again emphasized the role of the bark. Schulmann & Détouillon (1932) distinguished between chronic lichenified eczema of the hands, perhaps due to resin, and acute 'sylvestro-sensibilization' of face, hands and genitals. Conjunctivitis was often associated (Lagrange, 1923). Oak, beech, chestnut, poplar, acacia (*Robinia*), plane, elm and conifers could all cause dermatitis. At this point Senear (1933) reviewed the position in detail.

Tenchio (1948) took matters a stage further by getting positive patch tests with *Parmelia caperata*, a lichen he found growing on the bark of firs, larches and chestnuts. Ghillini (1954) reported dermatitis from the same lichen growing on oak and ilex. Le Coulant & Lopès (1956) found that other lichens and mosses also contained the allergen, but much the strongest reactions were obtained with *Frullania* and other liverworts of the family Jungermannales. These liverworts are found on the bark of most deciduous trees in S.W. France, and on undergrowth and rocks even among conifers, on the bark of which lichens prevail. In their review Le Coulant *et al.* (1966) concluded that liverworts were the main cause of woodcutter's eczema, and were in fact responsible for 2–4% of all contact dermatitis in that part of France. According to Philippe *et al.* (1965) they also cause rhinitis, asthma and urticaria, with positive intradermal tests. Knoche *et al.* (1969) showed that sesquiterpene lactones (Fig. 5) similar to those widespread in the Compositae were the sensitizers in *Frullania tamarisci*.

Mitchell (1965) investigated 'cedar-poisoning' in British Columbia lumbermen, obtaining positive patch tests with bark lichens and the usnic acid they contain, but negative results with the wood itself (Mitchell & Armitage, 1965). Champion (1965) found similar usnic acid sensitivity in an English woodcutter's wife who had only indirect contact with ash bark through her husband's working clothes. Since then he has seen patients with contact sensitivity to lichens who did not react to usnic acid. Several atopic patients had exacerbations of eczema as well as rhinitis and asthma after contact with lichens and algae, especially on apple trees. Three of them had positive patch tests with lichens (mainly *Lecanora* spp.), but algae gave only positive prick tests (Champion, 1971). Mitchell *et al.* (1969) now consider that in America as in Europe *Frullania* is a more frequent cause of dermatitis than lichens, and have studied cross-sensitivity between liverworts and Compositae (Mitchell *et al.*, 1970, 1972).

Cases of asthma caused by oak sawdust, with positive inhalation tests, were described by Scheven *et al.* (1963) and Sosman *et al.* (1969). The allergen was not identified and could have been either part of the wood itself or from *Frullania* on the bark. No satisfactory reports of asthma caused primarily by beech or chestnut sawdust have been found.

Three St John's patients gave positive patch tests with oak (*209*).

In Spain and Portugal workers with the bark of the cork oak (*Quercus suber* L.) suffer from suberosis, which starts with rhinitis, cough and dyspnoea and leads eventually to chronic bronchitic changes or extrinsic allergic alveolitis (Avila, 1972). The mechanism is unknown;

sensitivity to cork has not been demonstrated and fungus not found, but liverworts may not have been looked for. The cork contains much silica (Schepers, 1964; Cancella, 1955, 1963).

English woodworkers in the High Wycombe area have a much higher incidence of adenocarcinoma in the paranasal sinuses than the general population (Acheson *et al.*, 1968). There is nothing to indicate which sawdust, if any, is responsible, but beech is the most largely used.

ULMACEAE

25. *Ulmus procera* Salisb. et spp. **English elm**

Elm is one of the causes of 'woodcutter's eczema' (*24*). The leaves have irritant hairs on their under surfaces (Step, 1904; Genner & Bonnevie, 1938).

26. *Celtis brieyi* De Wild. Diania
C. cinnamomea Ldl. Gurenda

Diania is listed by Hublet *et al.* (1972) as irritating to skin or mucosae, without further details. Gurenda has an 'exceedingly unpleasant scent' (Gamble, 1902) and is used in medicine in Ceylon.

MORACEAE

27. *Ficus carica* L. et spp. Fig, peepul

The sap of many species irritates and photosensitizes (Berlin, 1930; English & Grey, 1943; Behl & Captain, 1966). The aerial roots are used locally for timber.

28. *Piratinera guianensis* Aubl. **Snakewood,** amourette (NP),
(*Brosimum guianense* Hub.) letterwood, tigerwood,
muirapenima, muskatholz

Working with this wood causes headache, irritation of the nose and throat, nausea and skin inflammation (Grossmann, 1920). In Brazil, Freise (1932) found that the sawdust produced salivation and severe thirst, and all parts of the tree were very irritating to the skin where it was thin. Freise rubbed a little bark-powder on his forearm and there was burning inflammation leaving a stain which persisted for some weeks. Hausen (1970) found evidence of quinones in the wood.

29. *Brosimum paraense* Hub. **Satine,** satiné rubané (NP),
(*Piratinera paraensis* Ducke) muirapiranga, satinwood, bois de
Féroles, partridgewood

Hausen (1970) found that this wood contained quinones similar to those in amourette (*28*) and also much saponin, but the clinical evidence against it is less satisfactory. Czimatis & Hagemann (1910) listed as toxic (without any reference) bois de Féroles, which is now known to be *B. parense* and not *Ferolia* sp. (Record & Hess, 1943). Freise's (1932) toxic 'muirapiranga' belonged to the Sapotaceae and was not *Brosimum.*

30. *Streblus brunoniana* F.Muell. Whalebone tree
(*Pseudomorus brononiana* Bur.)

The sap of this tough wood which is used by aborigines for boomerangs causes painful conjunctivitis (Maiden, 1920).

31. *Antiaris toxicaria* Lesch. Upas, ipoh

When gothic horror stories were in vogue at the end of the 18th century, travellers' tales exaggerated the powers of the deadly upas tree to such an extent that it was believed to devastate whole valleys, killing every animal in its shade and every bird that flew over it (Yule & Burnell, 1903; Oxford English Dictionary s.v. upas). Despite scientific scepticism the upas remained a symbol of venom until Malaya and Java became more familiar to Europeans through colonization and wars. The obsession with preternaturally toxic trees is still with us (Fleming, 1964).

The sap is in fact used as an arrow poison but it is harmless on unbroken skin (Gimlette, 1929). It contains a number of toxic glycosides including antiarin (Wehrli *et al.*, 1962).

Antiaris africana Engl. Antiaris, ako (NP), oro, ogiovu,
A. welwitschii Engl. kyenkyen, kirundu

Unlike the upas, these African species are exported commercially. Nigerian timber workers say their sawdust sometimes irritates the nose and skin (Woods, 1964), and they are careful not to let the latex touch scratches or skin eruptions (Irvine, 1961).

32. *Chlorophora excelsa* Benth. & Hook. f. **Iroko** (NP), mvule, odum, kambala,
C. regia A.Chev. moule, African teak

C. tinctoria (L.) Daub. **Fustic**, mora, tatajuba (NP)

Czimatis & Hagemann (1910) reported two outbreaks of dermatitis in a waggon factory where men were sawing and planing moule. The symptoms were worse in hot weather when they were sweating more. A similar clinical picture with pronounced swelling and itching of the eyelids and genitals, sometimes accompanied by coughing and sneezing, was described by Loro (1923). Frei (1932a) obtained positive patch tests with sawdust, and Ragot & Brun (1948) with an aqueous extract. Davidson (1941) in Britain and Thienemann (1941) in Germany described further outbreaks. In East Africa unseasoned iroko caused dermatitis in men who could tolerate properly seasoned wood (Piorkowski, 1944).

King & Grundon (1949, 1950) isolated from the wood chlorophorin, an oxystilbene with unsaturated aliphatic side-chain (Fig. 4), and Schulz (1957, 1962) proved that this was the main sensitizer in his seven cases. Chlorophorin 1% in vaseline is useful in diagnostic patch tests (Wilkinson, 1971). Another stilbene, maclurin, used to be extracted on a large scale from *C. tinctoria* for use as a dye.

Irritation by this widely-used wood is still often reported in the trade (FPRL, 1963; Orsler, 1969) despite exhaust ventilation and other precautions. It is officially recognized in Belgium as a cause of industrial asthma (van Ganse, 1968). Two cases of iroko dermatitis have been seen at St John's (*204*).

33. *Maclura pomifera* (Rafin) C.B.Rob (*M. aurantiaca* Nutt., *Toxylon pomiferum* Rafin) — Osage orange, bow-wood

The mucilaginous sap of this locally-used wood causes dermatitis (Schur, 1932). It contains tetrahydroxystilbene, which is very similar to chlorophorin (*32*) (Laidlaw & Smith, 1959).

URTICACEAE

34. *Cecropia* spp. — Imbauba (NP), guaramo, bois trompette, parasolier

Guaramo is used locally in Central and South America but not exported. Its sap and leaves cause dermatitis and it is an 'ant-tree', the hollow stems harbouring particularly vicious ants (Allen, 1943).

PROTEACEAE

35. *Grevillea robusta* A.Cunn. — Grevillea, silky oak (NP)

A native of Australia, but imported to the U.S.A. mainly from East Africa and called African silky oak there to distinguish it from *Cardwellia sublimis*.

May (1960) reported that a man developed erythema and bullae of the exposed skin and the genitals 2 days after sawing off a branch. A patch test with the sawdust gave a bullous response, but control subjects were not tested. Aborigines in Eastern Australia use the vesicant sap to scarify their bodies (Cleland, 1925). The flowers of *G. banksii* sensitize, but do not cross-react with those of *G. robusta* (Arnold, 1942). Several species contain 5-(10-pentadecenyl)resorcinol and other vesicant phenols very similar to those of poison ivy (Occolowitz & Wright, 1962; Ritchie *et al.*, 1965; Ridley *et al.*, 1968).

SANTALACEAE

36. *Santalum album* L. — **Sandalwood,** santal (NP)

No case reports of sandalwood dermatitis have been found. It is said to be toxic by Doerr (1913), Grossmann (1920), Hanslian & Kadlec (1966) and other authors, perhaps because the oil taken internally can produce eruptions. The oil has been found to irritate some animal skins experimentally (Opdyke, 1974). Not every so-called sandalwood or sandalwood oil comes from this species (Starke, 1967).

OPILIACEAE

37. *Agonandra brasileira* Miers — Pao marfim, granadillo

This satinwood substitute contains a saponin which irritates mucous membranes (Freise, 1932). The name pao marfim more often refers to *Aspidosperma vargasii* (*179*), which is also irritant.

POLYGONACEAE

38. *Coccoloba uvifera* L. Sea grape
C. laurifolia Jacq. Pigeon plum

Twenty workmen in Strasbourg were said to have irritation of the eyes and nose after working with coccoloba in 1904. As investigations into the cause were unsuccessful nothing was published at the time, but Czimatis & Hagemann (1910) refer to the incident. Hausen (1970) who elucidated this, has also heard of irritation by the wood in Guadeloupe, but there are no published case-reports. Hausen found several alkaloids in the wood.

The strong sensitizer cocobolo (*94*) was not finally identified as *Dalbergia* spp. till 1918, and was long confused with similarly named woods such as *Coccoloba*, *Cocos* and cocus (Wiesner, 1903). Cocobolo seems the likeliest cause of the Strasbourg outbreak, as *Coccoloba* has never been much exported (Boulger, 1908; Record & Hess, 1943).

BERBERIDACEAE

39. *Berberis vulgaris* L. Barberry

The wood is used to a limited extent for turning and inlaying, and may cause cramps, vomiting and diarrhoea. It contains berberine and other alkaloids (Hausen, 1970).

MENISPERMACEAE

40. *Coscinium fenestratum* Coleor. Colomboholz, false calumba
(*Menispermum fenestratum* Gaertn.)

The yellow wood contains up to 3·5% of berberine (*39*) (Perrins, 1852) and in Ceylon a dye and a bitter tonic are extracted from it. Vorreiter (1958) lists it as a toxic wood causing vomiting, diarrhoea and cramps, but the plant is only a climber and produces no timber (Gamble, 1902).

MAGNOLIACEAE

American whitewood

41. *Liriodendron tulipifera* L. Tulip tree, canary whitewood, poplar (U.S.)

Hausen (1970) described two cases of dermatitis of the hands and face from whitewood sawdust, one proved by patch tests, which were negative on ten eczematous controls. He found a labile quinone in the heartwood, but this did not appear to be the cause of the dermatitis. A sesquiterpene lactone has recently been discovered (Hausen, 1973). Nothing is known of the physiological activity of the isoquinolone alkaloid liriodenin, previously found in the wood.

MYRISTICACEAE

42. *Pycnanthus angolensis* Warb. **Ilomba** (NP)
Staudtia stipitata Warb. Niové (NP)

Hausen (1970) quoted trade reports that ilomba had caused nausea and vomiting, but he had no clinical details. Alemany-Vall (1958) lists niové as a skin-irritant.

LAURACEAE

43. *Phoebe porosa* Mez. (*Ocotea porosa* L.Barr.)	**Imbuya,** imbuia (NP), embuia, Brazilian walnut, laurel

Schmieg (1926) noted that imbuya sawdust was irritating to some workmen, and Schwartz (1931) reported eleven cases of vesicular dermatitis caused by it. The incidence varied from 10 to 20% in different factories, and many men without skin reactions had coryzal symptoms. Patch tests with moistened sawdust were positive on 'volunteers' (with dermatitis ?).

Imbuya (Brazilian walnut) was identified as *Nectandra* sp. by Schwartz and earlier writers, but Record (1929) showed that it was in fact *Phoebe porosa*.

44. *Ocotea rodiaei* Mez. (*Nectandra rodiaei* (Hook.) Schomb.)	**Greenheart** (NP), sipiroe, Guyana-, Demerara-, Antilles-greenheart

This very durable wood, exported for marine work since the eighteenth century, has sometimes been confused with the more irritating Surinam greenheart (*Tabebuia ipe 190*). Splinters of *O. rodiaei* cause troublesome wounds and the dust irritates workers' throats (Grossmann, 1920; Woods, 1951). It contains several alkaloids (McKennis *et al.*, 1956; Hearst *et al.*, 1968).

Ocotea barcellensis Mez.	**Louro inamui,** inhamui (NP), jigue
O. rubra Mez.	**Red louro,** louro vermelho (NP), determa, wana
O. puberula Nees.	Laurel amarillo

Many S. American species of *Ocotea* or *Nectandra* are called louro or laurel. Record & Mell (1924) describe the wood of *O. puberula* as peppery.

Five cases of dermatitis from louro have been seen at St John's (*206*).

Octoea usambarensis Engl.	**East African camphorwood**
O. bullata E.Mey	Stinkwood, S. African teak

The FPRL (1963) have had two trade reports of skin and mucous membrane irritation by *O. usambarensis*. Schwartz *et al.* (1957) list *O. bullata* as a cause of dermatitis, but quote no source.

45. *Nectandra* spp. (many are called *Ocotea* spp. by other botanists)

Freise (1932) divided the toxic Lauraceae of Brazil into three groups:

(a) Canella: *Nectandra* spp.

(b) Itauba: probably *Ocotea* spp., not *Mezilaurus* which is another itauba (Record & Mell, 1924).

(c) Pao cravo: *Dicypellium* (*46*)

According to Freise all these contain apiol and at least two alkaloids which cause vomiting, diarrhoea, cramps, cardiac stimulation, headaches and dizziness. Itauba also contains skatol.

Some *Nectandra* spp. from N. Brazil have a strongly irritant oil in their bark, which causes persistent pyoderma in the fingerwebs and at the angles of the mouth. See also *43*.

46. *Dicypellium caryophyllatum* (Mart.) Nees	Pao cravo, red wood, canella, Cayenne rosewood

The bark of this tree is used as a substitute for cloves and cinnamon, but the wood is little exported from Brazil. Freise (1932) classes it with the toxic *Nectandra* and *Ocotea* spp. (*45*).

47. *Aniba rosaeodora* Ducke	Bois de rose femelle (NP)
A. duckei Kosterm.	Pau rosa

The *Convolvulus* rhizomes and stem-bases called rhodorhiza (*186*) were the main source of oil of rosewood in the ninteenth century, and when they were replaced by *Aniba* wood the old name lignum rhodii (or rhodium) was applied to both. Unlike rhodorhiza, *Aniba* had been used for cabinet work under the name of Jamaican rosewood, but oil extraction eventually proved more profitable (Record & Hess, 1943).

Grossmann (1910, 1920) said that men in his father's workshop could not work for long with 'rosewood' because of the headache, weakness, drowsiness, dyspnoea and dermatitis it caused. He was aware that rosewoods came from many different sources, but thought the ones responsible for these symptoms were *Convolvulus* and *Amyris* (*105*). As the *Convolvulus* rhizome was never used as timber (Boulger, 1908) he was probably referring to *Aniba* wood, the source of which was then uncertain (Record & Mell, 1924). However, he went on to say that the *Dalbergia* rosewoods were innocuous, which we now know to be wrong (see *94*).

Grossmann's identifications are too shaky to support a case against *Aniba*, let alone *Convolvulus*. No other reports of toxic effects from either wood are known. Katz (1946) found in a series of patch tests on healthy subjects that rosewood oil caused at least one irritant reaction.

The wood contains linalool, anibine, cotoin and pinocembrine (Mors *et al.*, 1957; Gottlieb & Mors, 1958).

48. *Laurus nobilis* L.	Laurel, bay
Cinnamomum camphora Nees & Eberm. (*Laurus camphora* L.)	Camphorwood

Dermatitis from oil of laurel (leaves) was reviewed by Foussereau *et al.* (1967), who showed that the allergen was an unsaturated ketone. It may be a sesquiterpene lactone, as cross-sensitivity with *Frullania* has been reported (Asakawa *et al.*, 1974). Camphorwood contains camphor and borneol, and Grossmann (1920) experienced severe headache and giddiness after working with it. A man with dermatitis from cassia flavouring gave a positive patch test with oil of cinnamon leaves (*C. zeylanicum*) and a stronger reaction with cinnamic aldehyde (Kern 1960).

Camphor wood is sometimes used for chests and cabinets; laurel wood hardly at all (Hinckley, 1960).

49. *Cryptocarya pleurosperma* White & Francis	Poison walnut

Webb (1948b) described dermatitis starting on the hands 2 or 3 days after handling the cut bark or wood and later spreading to the face and the scrotum. He made no sawdust patch tests but reproduced the symptoms with the blistering alkaloid cryptopleurine isolated from the wood by Lalande (1948). Poison walnut is used a good deal in Australia despite its known toxicity.

50. *Umbellularia californica* Nutt.	Oregon myrtle, bay, Californian olive

The bark and leaves contain a pungent volatile oil (Record & Hess, 1943). According to Chittenden (1931) it affects some people like poison ivy.

HERNANDIACEAE

51. *Hernandia sonora* L.	Toporite, topolite
H. guinanensis Aubl. et spp.	Bois sabot (NP), mirobolan

Toporite was used in England for a time about 1950, but was found to cause dermatitis and has not been imported since (FPRL, 1963). It contains podophyllotoxin and picropodophyllin (King, 1953) (Fig. 7). Bois sabot is not exported from Central America.

CAPPARIDACEAE

52. *Crateva tapia* L.	Palo de guaco, garlic tree

The locally-used wood smells of garlic when first cut and its caustic sap produces a pronounced skin rash (Allen, 1943).

ALTINGIACEAE

53. *Liquidambar styraciflua* L.	**American red gum,** sweet gum, sap gum, hazel pine, bilsted, satin walnut, satinwood

Krais (1910) considered satin walnut irritant, but Legge (1907) and Grossmann (1920) were convinced that it was harmless and had been confused with irritant satinwoods such as *Fagara* (*103*). Nestler (1911) examined two specimens of 'East Indian satinwood' imported into Germany and found one was American satin walnut. An ether extract of the wood raised a painless blister on his arm, but the sawdust had no effect.

It is no longer much imported into Europe, but remains a major timber in the U.S.A. There are no reports of toxic effects there.

PLATANACEAE

54. *Platanus acerifolia* Willd.	**European plane,** London plane
P. orientalis L.	Oriental plane
P. occidentalis L.	**American plane,** buttonwood, sycamore (U.S.)

Plane is one of the trees that cause woodcutter's eczema (*24*). The down under the young leaves has been known since Galen's time to irritate the throat and eyes (Webster, 1920).

CHRYSOBALANACEAE

55. *Parinari guianense* Aubl. (*P. variegata*, *Parinarium* spp., *Ferolia* spp.)	Guyana or Antilles satinwood, atlaswood

The bois de Féroles from French Guyana, listed as *Ferolia* spp. by Czimatis & Hagemann (1910), was in fact *Brosimum paraense* Hub. (*29*) (Record & Hess, 1943). The toxic *Fagara flava* (*103*) used to be confused with *Ferolia* spp. (Grossmann, 1910).

There are no reports of toxic effects from wood properly identified as *Parinari*.

LEGUMINOSAE: MIMOSOIDEAE

56. *Albizia lebbeck* (L.) Benth. (*Albizzia lebbek*)	**Kokko,** siris, acle, pluk, E. Indian walnut, sneezewood
A. tetragona Willd. (*Calliandra tetragona* Benth.)	Mexican kieselholz, tendre à caillou
A. falcata Backer	jeungjing, djeungdjing, batai
A. ferruginea Benth. et spp.	**W. african albizzia,** ayinre, okuro

Many species of *Albizia* contain large quantities of saponin and are used as soaps and fish-poisons (Burkill, 1935). Those cut for timber produce nasal irritation and sometimes general malaise (Lewin, 1928; Vorreiter, 1958; Irvine, 1961). Griffioen (1949) described mucosal irritation from *A. falcata* in S. Africa, and the W. African species cause similar complaints (Wendorff, 1964; Farmer, 1972).

57. *Lysiloma latisiliqua* Benth. (*L. l. sabicu*, *L. sabicu*)	Sabicu (NP), jigue, horseflesh mahogany

Peltophorum adnatum Gris. is also called Cuban sabicu or sabicu moruro. Legge (1907) quoted a Manchester factory inspector's report that 'sabicu wood from Cuba' gave off 'a snuffy dust under the machine and hand planes, the effect of which upon the worker is to cause a running at the eyes and nose, and a general feeling of cold in the head. The symptoms pass off in an hour or so after discontinuance of work'. See *58*.

Virtanen & Gmelin (1959) found an alkaloid in *L. bahamensis* Benth.

58. *Pithecellobium arboreum* Urban (formerly *Pithecolobium*)	Bahama sabicu, black tamarind, turkey gill

Bahama sabicu was used from 1878 for bobbins in English cotton-mills (Record & Mell, 1924) and is another possible source of the sabicu mentioned above (*57*). Freise (1932) identified his irritant wood angelim (partridge wood) as *Pithecolobium* sp. but this seems improbable (Record & Hess, 1943). Schwartz *et al.* (1957) quote no source for their listing of *P. arboreum* and *P, filicifolium*.

59. *Samanea dinklagei* Keay

The pounded bark is a soap substitute, and the dry sawdust irritates the nose and throat (Irvine, 1961). The timber is not exported from W. Africa.

60. *Acacia melanoxylon* R.Br. **Australian blackwood**
A. harpophylla F.Muell. Brigalow
A. shirleyi Maiden Lancewood

Workers in joinery shops and motor-body factories sometimes developed severe itching, weeping dermatitis on the forearms, neck and face after using Australian blackwood (Cleland, 1925). Cleland found that patch tests on himself were negative and suggested that sensitization must play a part. Nott (1925) had a papular rash on his own hands and forearms after working with blackwood.

'Brigalow itch' affects men ringing the trees and is probably due to a fine yellow powder on the bark, but irritant bag-moth shelters and cocoons are found on several related species (Cleland, 1925). Splinter wounds from *A. shirleyi* and other species are often troublesome and persistent (Maiden, 1919; Webb, 1948a).

Acacia wood contains flavonols, stigmasterol, saponins, etc. but the irritant has not been identified (Hausen, 1970).

61. *Prosopis juliflora* (Sw.) D.C. Mesquite, mezquit

Stewart (1940) reported a case of dermatitis of the face in a man working on a cabinet made of mesquite, and obtained a positive patch test with the sawdust. Fox (1941) described extensive dermatitis in a farmer who regularly sawed up the wood for a stove, and found patch tests with the moist sawdust and an ether extract were positive. The wood is used only locally in the southern U.S.A. and Central America. Hausen (1970) found evidence of an alkaloid in it (not prosopinine, an irritant alkaloid found in the leaves and perhaps the wood of *P. africana*).

62. *Piptadeniastrum africanum* Brenan **Dahoma,** ekhimi, agboin,
(*Piptadenia africana* Hook. f.) dabéma (NP), mpewere

Freshly sawn wood has a strong ammoniacal smell, which disappears on seasoning. Irritation of the eyes and nose by its sawdust is well known to timber workers in Africa (Irvine, 1961; Wendorff, 1964) and elsewhere (Anon., 1923; Nomenclature générale, 1965), but skin irritation is less common (FPRL, 1963; Farmer, 1972). All the thirteen men working with dahoma in one English factory complained of severe irritation of the nose, throat and trachea (MacKenna & Horner, 1954).

63. *Xylia xylocarpa* Taub. **Pyinkado** (NP)
(*X. dolabriformis* Benth.)

The sawdust causes sneezing (Laslett, 1894; Boulger, 1908).

64. *Plathymenia reticulata* Benth. **Vinhatico,** v. castanho,
Echirospermum balthazarii Fr.All. v. espinho

The sawdust of vinhatico contains saponins which are very irritating to the nose, conjunctiva and other mucous membranes (Freise, 1932). Freise identified it as *E. balthazarii*, but according to Record & Mell (1924) *P. reticulata* is the more likely source in Brazil. Yet another vinhatico is *Chloroleucon vinhatico* Record, from Honduras (Nomenclature générale, 1965).

LEGUMINOSAE: CAESALPINIOIDEAE

65. *Erythrophleum guineense* G.Don.
E. ivorense A.Chev.
Missanda, tali (NP), sasswood, erun, red-water tree

The bark of sasswood contains several alkaloids with digitalis-like action and has long been used in W. Africa for judicial ordeals and as a fish-poison. Men preparing the bark suffer from coryzal symptoms (Cornevin, 1887; Irvine, 1961). Lewin (1928) said that an extract of the wood caused vomiting, respiratory disturbance, bradycardia, headache and vertigo, and anaesthetized mucous membranes by contact, but Hausen (1970) found alkaloids only in the bark and not in the wood. The FPRL (1963) receives a number of trade complaints about irritation of the nose and eyes by the wood, and troublesome splinter wounds.

E. laboucheri F.v.M. in Australia and *E. coumiga* H.Bn. in Madagascar are probably also toxic (Hausen, 1970; Golse, 1937).

66. *Copaifera bracteata* Benth.
Copaiba

This was formerly thought to be the source of the wood called purpleheart or amarant (Stone, 1924; Gayer, 1928), toxic effects from which were described by Grossmann (1920). Purpleheart is now known (Record & Hess, 1943; Nomenclature générale, 1965) to come from *Peltogyne* spp. (*68*). *C. bracteata* produces no significant timber but only copaiba gum.

67. *Hymenaea courbaril* L. et spp.
Courbaril (NP), copalier, algarrob, W. Indian locust

The wood causes skin irritation (Freise, 1932). Copal resin is obtained from this tree.

68. *Peltogyne densiflora* Spruce et spp.
Purpleheart, amarante (NP), pau roxo, bois violet, blue wood

Purpleheart, formerly thought to come from *Copaifera* (*66*), has a very unpleasant smell when worked and causes nausea and loss of appetite (Grossmann, 1920). The dust from working and polishing it causes cough and sometimes nettlerash in Brazil (Freise, 1932). De Sousa *et al.* (1967) found the known allergen 4-methoxydalbergione (*94*) in the heartwood of *P. densiflora* and of *Goniorrhachis marginata* Taub. (guarabu, itapicuru). No toxic effects have been reported from guarabu.

69. *Afzelia africana* Smith et spp.
Afzelia, doussié (NP), apa, aligna, chanfuta, lingue

In West Africa there are frequent complaints of skin and nasal irritation from afzelia (Irvine, 1961; Woods, 1964), and after an early trial at the Imperial Institute in London it was reported to be 'very unpleasant to work owing to its dust causing sneezing' (Anon, 1923). Trade complaints are not common now in England, however (Orsler, 1969), though they occur in France (Raymond, 1959). Afzelia contains kaempferol and other catechins (King & Acheson, 1950; King *et al.*, 1955).

70. *Distemonanthus benthamianus* Baill.
Ayan, movingui (NP), anyaran, Nigerian satinwood

Morgan & Thomson (1967) reported the case of a man who had several attacks of dermatitis after exposure to shavings and sawdust of ayan. Patch tests were positive with sawdust and with oxyayanin A and B extracted from it, but hardly at all with its other flavonoids ayanin and distemonanthin (isolated by King *et al.*, 1954). Some specimens of ayan contain more oxyayanin B, some more distemonanthin, and there are corresponding variations in their sensitizing capacity. Oxyayanin A has recently been found also in *Apuleia leiocarpa* (*71*).

Thirteen cases of ayan dermatitis have been seen at St John's (*199*).

71. *Apuleia molaris* Spruce (*A. leiocarpa* MacBride, *A. ferrea* Mart.) — Ferro (NP), muirataua, redwood, garapa

Some botanists (but not Record & Hess, 1943) separate *A. molaris* (muirataua) from the more widespread *A. leiocarpa* (garapa).

The toxic action of this wood persists for years after it is dry, and workers once affected usually become hypersensitive to it (Freise, 1932). A German carpenter developed headache, vertigo, visual disturbances, thirst and dyspnoea after contact with the fresh sawdust, especially on hot days (Freise, 1936). He later had increasingly severe dermatitis on the hands and arms. Freise found in the wood the cytisine-like alkaloid phellandrene, a saponin, and two red dyes which seemed to be responsible for the dermatitis. More recent workers have identified flavonoids including the known allergen oxyayanin A (Braz Filho & Gottlieb, 1968)—see *70*.

The nomenclature of S. American timbers is always obscure, but Freise (1936) has muddied the waters very thoroughly here. He heads the section on *A. molaris* 'Cocoboloholz' but later explains that it is not cocobolo but a substitute for it; then he calls it the 'Caesalpiniacee apuleia molaris Benth', which has misled some later authors into placing it in a non-existent genus *Caesalpiniacea*.

72. *Cassia siamea* Lamk. — Djoher (NP), muong ten, tagayasan, bois perdrix

Iwakawa (1911) studied the conjunctivitis, keratitis and iritis, sometimes accompanied by dermatitis with brown staining of the skin, which affected Japanese furniture workers using this wood. Cracks in the wood contain a yellow powder darkening to violet on exposure, in which he found the irritant anthranol of chrysophanic acid (see *97*).

73. *Koompassia excelsa* Taub. — **Kempas** (NP), tualang

The sap is said in Malaya to irritate the skin, producing an inflammation like erysipelas (Burkill, 1935). Titmuss (1965) comments on the pungent smell of the wood when fresh.

74. *Dicorynia guianensis* Amsh. (*D. paraensis* Benth.) — **Basralocus** (NP), angélique

This teredo-resistant wood contains much silica and two alkaloids, one of which is tryptamine (Sandermann & Lange, 1967). There is no clinical evidence against it, however, except a casual remark by Baumer (1955) that it is 'reputed toxic'.

75. *Haematoxylum campechianum* L. — Logwood, campeche

The dye haematoxylin used to be extracted on a large scale from logwood. Hausen (1970) quotes an unconvincing case of dermatitis from the sawdust in 1912 (without patch tests), and Sézary & Deval (1932) described dermatitis of the hands said to be due to haematoxylin dye in gloves. Patch tests were positive with the glove but not done with the dye.

76. *Caesalpinia echinata* Lam. — **Brazilwood,** pernambouc (NP),
(*Guilandina echinata* Spreng.) — fernambouk, Bahia wood, para wood, pau Brasil, ibiripitanga
C. paraensis Ducke — Muirapixuna

According to Record & Hess (1943), muirapixuna is *Swartzia tomentosa* DC.

Freise (1932) includes these among the Leguminosae that cause skin irritation. The nomenclature is very confused.

77. *Microberlinia brazzavillensis* A.Chev. — **Zebrano,** zingana (NP), amouk,
M. bisulcata A.Chev. — African zebrawood

Zafiropoulo *et al.* (1968) list this wood as irritant but quote no source. *Astronium* spp. (*137*) and other known irritant woods are also called zebrawood.

78. *Batesia floribunda* Benth. — Acapú rana (NP)
Campsiandra laurifolia Benth.

Both these woods are called acapú rana (false acapú) or manaiara according to Freise (1932), who says that men using mechanical saws on them suffer from headaches and sometimes vomiting.

79. *Melanoxylon brauna* Schott — Braúna (NP), grauna

Freise (1932) describes pustules and necrotizing inflammation caused by the sawdust of braúna, especially when it contaminates minor wounds on the legs. The seedpods contain an alkaloid with cardiac and respiratory effects.

80. *Zollernia paraensis* Hub. et spp. — Pau santo (NP), mocitahyba

Freise (1932) names pau santo as one of the Leguminosae that cause irritating swellings of the skin.

LEGUMINOSAE: PAPILIONOIDEAE

81. *Castanospermum australe* A.Cunn. — **Black bean,** Australian or Moreton Bay chestnut

Black bean has been 'accused of injuriously affecting the health of workmen' in Australia, where it is used for high-class joinery (Maiden, 1909). It contains saponins, a phenol, and flavonoids including afrormosin (Eade *et al.*, 1963). Greshoff (1909) blamed saponins for the irritating effect of its sawdust on mucous membranes.

82. *Bowdichia nitida* Benth. et spp.
Sucupira, coeur dehors (NP), black sucupira, zwarte kabbes

Diplotropis purpurea Amsh. et spp.
(*D. guianensis* Benth., *B. guianensis* Ducke)
Sucupira, s. assu, sapupira, coeur dehors (NP)

Yellow sucupira is *Ferreirea spectabilis* (*87*).

Freise (1932) included sucupira among the irritant Leguminosae. Heyl (1966) reported the case of a joiner who developed dermatitis on the fingers, eyelids, elbow flexures and dorsa of the feet (working in sandals) whenever he used sucupira or palisander (*Dalbergia nigra*). Patch tests with both woods were strongly positive, and negative on nine controls. Hausen (1970, 1972) found that Heyl's sucupira was *B. nitida* and isolated three labile quinones from it, but could identify only the least active (2,6 dimethoxy-p-benzoquinone). The patient gave positive patch tests with all three, and also with dalbergiones.

83. *Pericopsis elata* van Meeuwen
(*Afrormosia elata* Harms.)
Afrormosia, kokrodua (NP), asamala, obang, oleo pardo

The FPRL (1963) receives occasional trade reports of skin and respiratory irritation (Orsler, 1969), and it is listed by Gronemeyer & Fuchs (1967) as a cause of asthma. Splinter wounds from afrormosia are said to be troublesome and often become septic (Wilkinson, 1971). In Germany there are trade reports of cramps and paralytic symptoms which may be due to the N-methylcytisine it contains (Hausen, 1970).

Two St John's patients gave positive patch tests with afrormosia (*197*).

84. *Myroxylon balsamum* Harms.
Balsamo (NP), quina-quina, oleo vermelho, cabreuva vermelha

There are two varieties: *balsamum* (*M. toluiferum* H.B.K.) which yields balsam of Tolu, and *pereirae* (*M. peruiferum* L.f.) giving balsam of Peru. Dermatitis from balsam of Peru (Opdyke, 1974) was investigated by Hjorth (1961), but there are no reports of reactions to the timber. It probably comes more from the *balsamum* variety (Record & Mell, 1924).

85. *Sophora japonica* L.
Pagoda tree, yen-ju

S. tetraptera J.Mill.
Kowhai

The bark of the pagoda tree has a strong smell which affects those who prune it. Four turners had colic and diarrhoea after working with freshly cut wood, but the dry wood is said to be harmless (Du Hamel, 1811; Loudon, 1844). It is little used outside China and Japan. Two people were seriously ill after eating from spoons made of kowhai wood (Aston, 1923). The seeds of *Sophora* spp. contain cytisine and related alkaloids (Briggs, 1946).

86. *Baphia nitida* Afzel.
Camwood

The sawdust is very pungent, like snuff (Holtzapffel & Royle, 1843). A red dye used to be extracted from the wood, but it is hardly used as timber. The related nyala wood (*Xanthocercis*, formerly *Pseudocadia zambesiaca* Harms.) is also a strong mucosal irritant (Codd, 1951).

87. *Ferreirea spectabilis* Fr.All. Lapachin (NP), manisito, yellow sucupira

King *et al.* (1952) found chrysophanic acid anthranol in this little-exported S. American timber, but Simatupang *et al.* (1967) could not confirm this. They suggested that King's material came from *Vatairea lundellii* (*98*), which is microscopically similar.

88. *Laburnum anagyroides* Medic. **Laburnum**
(*L. vulgare* Gris., *Cytisus laburnum* L.)

The wood of laburnum, like the notoriously poisonous seeds, contains the alkaloid cytisine (Cornevin, 1887). According to Lewin (1928) the sawdust causes salivation, sore throat, vomiting, headache and muscle pains, oliguria and faintness, but he quotes no cases or references.

89. *Robinia pseudoacacia* L. **Robinia,** acacia, false acacia, black locust

Acacia is one of the trees that cause woodcutter's eczema (*24*) in France (Schulmann & Détouillon, 1932). Wood (1957) mentions 'the strong dislike of the woodman for its fearsome thorns'.

90. *Millettia laurentii* De Willd. **Wenge** (NP)
M. stuhlmannii Taub. **Panga-panga**

Splinters of wenge lead to persistent wounds, and the sawdust causes abdominal cramps. Hausen (1970) found a quinone (perhaps a mixture) in the wood but could not sensitize guinea-pigs with it, or persuade the splinter patients to submit to patch tests. Hublet *et al.* (1972) list wenge among timbers causing mucosal or cutaneous irritation in Belgium but give no details.

91. *Brya ebenus* DC. **Cocuswood** (NP), cocus, granadillo, brown ebony, green e., Jamaican e.
(*Amerimnum ebenus* Sw., *Inga vera* Willd.)

During the nineteenth century cocuswood replaced boxwood as the favourite wood for flutes, though it was known to cause irritation of the lip in some players (Rockstro, 1890). Stern (1891) reported a case of persistent dermatitis of the lip and chin from a flute made of grenadilla or red ebony, which was almost certainly Cuban cocuswood. No patch tests were made, but the dermatitis cleared up as soon as the player changed to an ivory mouthpiece. Crocker (1903) mentioned dermatitis in flute-makers sawing up cocus wood. Thompson (1914) described dermatitis of the face due to 'cokus', which he distinguished clearly from 'coca-bola' (*94*). Some authors have confused cocus not only with cocobolo but even with the completely unrelated coconut palm (*Cocos*).

Schröpl (1935) described an unconvincing case of dermatitis in an instrument-maker who had not been in direct contact with cocus wood but gave a positive patch test with it.

Hausen (1970) found at least three quinones in the heartwood but could not identify the sensitizer.

92. *Piscidia erythrina* L.	Jamaica dogwood

The wood is a fish-poison, and according to Lewin (1962) also toxic to humans. It is little used as timber and no reports of ill-effects have been found.

93. *Pterocarpus indicus* Willd.	**Amboyna,** padauk (NP), padouk, New Guinea rosewood, narra, bija
P. santalinus L.f.	Red sandalwood, padauk (NP), santal rouge, red sanders
P. angolensis D.C.	**Muninga** (NP), kejaat, kiaat, African padauk, mbila, girassonde

Nestler (1924, 1926) quoted a statement in a German trade journal of 1910 that padouk wood from the Sunda islands (Indonesia) caused painful swelling and redness of the face in men working with it. He obtained two different specimens called padouk wood, neither of which could be identified botanically, though one resembled *P. indicus*. Ether extraction of this wood produced a red essential oil which caused irritation of his skin in less than 2 h, but the sawdusts of both woods and all other extracts of them gave negative patch tests.

Two Hamburg sawmill workers complained of an unpleasant sweet taste in the mouth, repeated vomiting and conjunctival irritation after contact with Ceylon mahogany, thought to be *P. santalinus* (Hausen, 1970). Other woods, such as *Artocarpus integrifolia* L., have also been called Ceylon mahogany (Boulger, 1908).

Ordman (1949) described two cases of asthma in men working with *P. angolensis*, one of whom was also sensitive to *Thuja plicata* (*13*) and a Congo hardwood identified by Schweisheimer (1952) as *Lovoa klaineana*. Intradermal tests with an aqueous extract of kejaat were positive.

Pterocarpus spp. contain pterostilbene and other fungicidal substances (Fig. 4), but these have not been shown to be toxic to man (Bevan, 1958).

94. *Dalbergia retusa* Hemsl. *D. granadillo* Pitt. (*Amerimnon granadillo* Standl.) *D. hypoleuca* et spp.	**Cocobolo** (NP), granadillo, nambar, palo negro, foseholz, red foxwood, rosewood

Nestler (1912) investigated a sample of cocobolo which had been sent to him because it caused dermatitis in workers. He obtained strongly positive patch tests on himself and other subjects with moist sawdust, and with alcohol and benzol extracts. Mackee (1913) described in detail dermatitis of the hands and face in men sandpapering cocobolo hairbrushes. Its irritant properties were already well known in the trade.

At that time there was much uncertainty about the origin of cocobolo, which was confused with *Coccoloba* (*38*), cocus (*91*) and other woods, but Pittier established in 1918 that it came from the above Central American *Dalbergia* species (Record & Hess, 1943). Record & Garratt (1923) wrote a monograph on cocobolo, clarifying its botany and quoting accounts of dermatitis from many American firms. The incidence was from 10 to 30%. The eruption began on the face and hands, and in severe cases became generalized with conjunctivitis and sometimes headache. Only men exposed to the fine dust from sawing, boring or sanding were affected, and then mainly in hot weather. Extractors were helpful but did not completely prevent trouble.

Cases of localized dermatitis from contact with knife-handles, bracelets, recorders, and other finished articles made of cocobolo were reported by Levin (1941), Leider & Schwartzfeld (1950), and Howell & Blair (1950). Many recorders were made of cocobolo in Germany about 1930, but they caused dermatitis of the mouth so often that the use of the wood had to be abandoned (Anon., 1933; Steinbrinck, 1950; Hausen, 1970). Meister (1934) described two cases of severe swelling of the eyelids with conjunctivitis, brought on by playing cocobolo recorders. The lips were not affected, though he had heard of a third case confined to the mouth. No patch tests were made.

Eaton (1973) reported two cases of nasal irritation in workers finishing and sanding cocobolo, with positive prick and patch tests (but no controls). One also had asthmatic symptoms. Some improvement was claimed after desensitizing injections. A third case was not definitely connected with cocobolo.

D. retusa contains S-4′-hydroxy-4-methoxy dalbergione, R-4-methoxy dalbergione and other quinones and phenols (Schulz & Dietrichs, 1962; Hausen, 1970).

Dalbergia nigra Fr.All. *D. cubilquitzensis* Pitt. *D. spruceana* Benth.	**Brazilian rosewood,** jacaranda, palissandre Brésil (NP), Rio or Bahia rosewood, caviuna, palisander, jacaranda pardo

Gougerot & Blamoutier (1922b) described eczema of the face and hands in a cabinet-maker working with palissandre, the source of which was not stated. Patch tests were positive with palissandre dust and an alcoholic extract of it, and negative with walnut and mahogany which he was also using. Application of increasing amounts of the extract to the patient's skin over a period of 11 days was said to produce successful desensitization. Gougerot & Delay (1932) reported another case of palissandre dermatitis, associated with lacrimation, sneezing and coughing. Touraine *et al.* (1934) identified the 'palissandre des Indes' in their case as *D. nigra*, but their botanical remarks are not impressive and its origin suggests *D. latifolia* (see below). Tottie (1938) described three cases of dermatitis in carpenters using Jacaranda envaco, which is a Brazilian name for *D. nigra*, not to be confused with the unrelated botanical genus *Jacaranda*.

In Brazil itself Freise (1932) described dermatitis in forestry workers handling various kinds of jacaranda, and Barroso (1947) wrote about dermatitis from caviuna, identified as *D. nigra*. He extracted a resin from the sawdust which gave positive patch tests on the patient, and sensitized rabbits and guinea pigs with it experimentally. Gronemeyer & Fuchs (1957) list *D. nigra* among the timbers which cause asthma. It contains R-4-methoxy-dalbergione and other quinones (Schulz & Dietrichs, 1962).

D. nigra is now in short supply, and the more irritant *Machaerium scleroxylon* (*95*) is sometimes substituted for it (Eyton *et al.*, 1965).

Dalbergia latifolia Roxb.	**Indian rosewood,** E. Indian r., Bombay blackwood
D. sissoo Roxb.	**Sissoo,** shisham
D. oliveri Gamb.	**Burma tulip wood,** pinkwood, bois de rose
D. greveana Baill.	Madagascar rosewood, French r.
D. cochinchinensis Pierre	Thailand rosewood, chin chan

The international name for all these (and more) species from Asia and Madagascar is Palissandre Asie (NP).

Sternberg (1908) described the urticarial rashes and swellings he had seen some years earlier in carpenters working with Indian rosewood. Escartefigue's (1935) patient developed dermatitis under a necklace and bracelet of Madagascar palissandre, but he made no patch tests. Some of the French cases mentioned under *D. nigra* may have been due to Asian palisanders. Findley (1972) reported disabling dermatitis in a man who had only indirect contact with *D. latifolia* sawdust.

D. latifolia and *D. cochinchinensis* contain R-4-methoxy dalbergione and other quinones (Dempsey & Donnelly, 1963; Donnelly *et al.*, 1968). Some related species which do not provide timber are used as fish-poisons (Lewin, 1928).

Dalbergia stevensonii Standl.

Honduras rosewood, nagaed wood, palissandre Honduras (NP)

Three trade reports of dermatitis from this wood (used particularly for xylophones) were known to the FPRL (1963). It contains 4-methoxy dalbergione (Hausen, 1970).

Dalbergia melanoxylon Guill. & Perr.

African blackwood (NP), mpingo, Mozambique or Senegal ebony, ébène, African grenadilla

Clarinets are usually made of African blackwood in England, and it is often used for flutes and other instruments, especially in Germany. Moll (1950) mentioned that it was known in the trade to be irritant. Two cases of dermatitis in clarinet-makers have been seen at St John's (*201*).

D. melanoxylon contains several quinones including S-4′-hydroxy-4-methoxy dalbergione (Donnelly *et al.*, 1969), and S-4-methoxy dalbergione (Hausen, 1970) which is also found in *D. violacea* (Eyton *et al.*, 1965).

Dalbergia variabilis Vog.
D. oliveri Gamb. et spp.

Brazilian tulipwood, bois de rose (NP)

D. cearensis Ducke

Kingwood bois de violette (NP), violet wood, violetta

There are no reports of toxic effects from tulipwood, which was formerly thought to belong to *Physocalymma* (Lythraceae).

D. cearensis is probably the 'kingswood' which Freise (1932) called *Machaerium violaceum* Ducke (Record & Hess, 1943). He described it as a very severe skin-irritant, often leading to persistent ulceration.

Chemistry of Dalbergia spp.

Earlier workers, summarized by Sandermann & Barghoorn (1956), blamed flavones, saponins and glycosides for the irritant effects of rosewood, but the discovery of sensitizing quinones in other woods such as teak and mansonia led Schulz & Dietrichs (1962) to look for similar substances in *D. nigra* and *D. retusa*. They found three quinones which they called Dalbergiaquinones A, B and C, and demonstrated by patch tests on patients that these were the sensitizers, the strongest being R-3,4-dimethoxydalbergione. Eyton *et al.* (1965) have studied the chemistry of this 'neoflavanoid' group of quinones in detail (Fig. 3). They have now been found in other *Dalbergia* spp., *Machaerium* (*95*), *Peltogyne* (*68*) and *Goniorrhachis* (*68*) (Hausen, 1970).

Ten patients sensitive to rosewood from *Dalbergia* spp. have been seen at St John's (*201*, *213*).

95. *Machaerium scleroxylon* Tul.	Caviuna vermelha, caviuna, jacaranda caviuna, pao ferro

Occasional logs of this inferior and more irritant substitute have always found their way into shipments of Brazilian rosewood (Record, 1926), but with the current popularity of rosewood and increasing scarcity of *D. nigra* the proportion is likely to increase (Phillips, 1966; Farmer, 1972). Morgan *et al.* (1968) described an outbreak of dermatitis in a furniture factory where it was unwittingly substituted for *D. nigra*. Seven of the twenty-four men exposed developed dermatitis on the hands, face and neck. Patch tests produced very strong reactions to *M. scleroxylon* sawdust with milder reactions to *D. nigra*, and the sensitizers were shown to be R-3,4-dimethoxy dalbergione and its related quinol (Fig. 3). Cases have also been reported in Brazil (de Miranda Bastos & de Matos Filho, 1960) and in Hamburg (Hausen, 1970), where patch tests were positive with the sawdust and with R-3,4-dimethoxy dalbergione.

Machaerium violaceum Vog.	Orelha d'onça, kingswood (?)
Paramachaerium schomburgkii Ducke (*Machaerium schomburgkii* Benth.)	Jacaranda, ximbo

The status of these woods as irritants is doubtful. Freise (1932) listed *M. violaceum* Ducke, but his name orelha d'onça is not found elsewhere and the usual source of 'kingswood' is *Dalbergia cearensis* (*94*). *M. violaceum* was, however, regarded as one source of Brazilian rosewood at that time (Howard, 1934).

M. schomburgkii seems to have been taken by Hanslian & Kadlec (1966) from Grossmann (1910), but he only mentioned it as one of the less likely sources of snakewood, which is now certainly known to be *Piratinera guianensis* (28).

96. *Andira inermis* H.B.K.	**Angelin** (NP), angelim, kuraru, partridge wood, cochenilla
A. coriacea Pulle	Saint Martin rouge (NP), angelin, red cabbage tree, mocha colorado

Lewin (1928) said that angelim was a skin-irritant. Freise (1932) described persistent cough and urticarial eruptions in those working with angelim (partridge wood), which he identified improbably as *Pithecolobium* sp. Ducke. The bark and seeds contain a poisonous alkaloid (Allen, 1943) and the wood contains demethylpterocarpin, which is an isoflavanoid similar to the strongly allergenic dalbergiones, and biochanin A which is related to ayanin (*70*) (Cocker *et al.*, 1962).

Simatupang *et al.* (1967) examined the very similar *A. coriacea* for irritant anthranols but found none (see *98*).

97. *Vataireopsis araroba* Ducke (*Andira araroba* Aguiar) — Angelim amarello, a. amargoso, a. araroba, faveira, araroba

The sawdust irritates the eyes of workmen (Record & Hess, 1943). Goa powder, found in the bark and wood, is the source of chrysarobin, the active principle of which is chrysophanic acid anthranol (Simatupang *et al.*, 1967). Before the introduction of dithranol it was a standard treatment for psoriasis (Trousseau, 1886; Pautrier, 1904). The timber is not exported.

98. *Vatairea guianensis* Aubl. — Quassia wood, gele kabbes, partridge wood

This wood caused several cases of dermatitis and conjunctivitis when it was imported into Hamburg incognito among other S. American timbers (Simatupang *et al.*, 1967). It was found to contain chrysophanic acid and physcion, and their anthranols which are the irritants as in chrysarobin. *V. lundellii* contains only chrysophanic acid (see *87*).

99. *Coumarouna odorata* Aubl. (*Dipteryx odorata* Willd.) — Tonka (NP), cumarú, tonquin bean

'On being sawed into thin slabs it gave off a most unusual quantity of bright red sawdust which covered the workmen. One of them, thinking to wash the dust from his hair, dipped his hair into a bucket of water, when, to his surprise, the hair turned a bright grass-green colour. It took him several days of scrubbing to restore the natural colour' (Thompson, 1914). In a brief trade journal report (Anon, 1906) twenty-six men in a Cambridge (Mass.) furniture factory were said to have been 'poisoned' by handling tonquin wood from the Philippines.

Coumarin is obtained commercially from tonquin beans and probably also occurs in the wood, which sometimes smells of vanilla (Record & Hess, 1943).

100. *Vouacapoua americana* Aubl. et spp. (*Andira excelsa* H.B.K.) — Wacapou (NP), acapú, tatbu, sapupira, partridge wood

Freise (1932) describes acapú as causing persistent itching papules. Cocobolo is not this wood (as Freise suggested) but *Dalbergia* spp. (*94*).

101. *Gossweilerodendron balsamiferum* Harms. — **Agba,** tola (NP), tola branca, Nigerian cedar

A few cases of skin irritation have been reported to the FPRL (1963) (Orsler, 1969), and two have been seen at St John's (*198*). The wood is extensively used, however, and the incidence of reactions probably low. Symanski (1957) mentions asthma caused by the sawdust of 'tola', which was probably agba rather than the less common tchitola (*Oxystigma oxyphyllum* Léon.)

ZYGOPHYLLACEAE

102. *Guaiacum officinale* L. et spp. — **Lignum vitae,** gaiac (NP), guayacan, pockholz

The sawdust causes sneezing, especially that of *G. arboreum* D.C., which a French workman called *pique-nez* (Guibourt, 1849). The FPRL (1963) had two trade reports of mucosal irritation. Frei (1932b) described a case of dermatitis which began round a minor finger wound contaminated with lignum vitae sawdust, and spread to the hands and arms. Patch tests were positive with lignum vitae and negative with mahogany, walnut, palisander and teak. Control subjects gave negative patch tests.

RUTACEAE

103. *Fagara flava* Krug. & Urb. (*Zanthoxylum flavum* Vahl.)	**West Indian satinwood,** Jamaican or San Domingan satinwood, espenille (NP), yellow sanders, atlaswood, citronwood, zitronenholz

Botanists have still not agreed whether to place this species in *Fagara* or *Zanthoxylum*, but seem determined to keep the curiously illiterate spelling of the latter in preference to *Xanthoxylum* which was once commoner. Several other species of these and related New World genera provide satinwoods of poorer quality, some of which have been described as toxic (Smith, 1920; Lewin, 1928). See *104* below. Many of the vernacular names given above are highly non-specific.

Grabham (1905) wrote that dermatitis from satinwood (mainly *F. flava*) was well known among labourers in the West Indies; he had had it himself. Wechselmann (1909) gave a clear description of dermatitis in a joiner using *F. flava*, with a positive patch test with the sawdust of this wood only, and suggested an 'anaphylactic' mechanism. Siegheim (1909) and Volk (1911) investigated three more cases of satinwood dermatitis in which the identification of the wood was less satisfactory and the patch tests equivocal. There are many other reports of dermatitis from satinwood, atlaswood, citronwood or zitronenholz, which may or may not have been *Fagara* spp. (Brezina, 1913; Oppenheim, 1909; Noll, 1931). *F. caribaea* is one of a number of woods called zebrawood (*137*) and not the only one likely to cause dermatitis. See also *108*.

Fagara macrophylla Engl.	**African satinwood,** olonvogo (NP), olon dur, bahé, bongo, lisumba
F. heitzii Aubrev. & Pellegr. *F. inaequalis* Engl.	Olon (NP), bongo, mbanza

Pillon & Martinez (1935) reported an outbreak of severe dermatitis and coryza affecting all the eight men present when a very dry trunk of olon was sawn up producing much fine dust. A damper section of the same trunk was sawn elsewhere without any trouble. Rochaix & Vieux (1936) tried to sensitize rabbits experimentally with olon sawdust, but could demonstrate only a primary irritant effect. The bark is vesicant and is used in arrow poisons (Irvine, 1961). The FPRL (1964) has received a few reports of irritation from olon in England, and Hausen (1970) mentions a trade report of skin irritation by olonvogo in Hamburg.

The early work on the chemistry of *Fagara* spp. reflected the contemporary bias towards alkaloids. Wechselmann (1909) found chloroxylonine (see *108*) in *F. flava* and obtained positive patch tests with it on his patient and himself, but not on two other people. Auld & Pickles (1912) found only pungent resins, while Freise (1937) found xanthotoxin and an alkaloid. More recent work has demonstrated suberosin and other coumarins, psoralene and xanthotoxin in *Fagara* and xanthyletin in *Chloroxylon* (King *et al.*, 1954).

Wilkinson (1971) has seen a case of clinical photosensitivity to *Chloroxylon swietenia* (*108*) but this could not be confirmed experimentally. Photosensitization is characteristic of the Rutaceae (Woods, 1962), and if it does prove to be the mechanism of timber dermatitis in this family many of the inconsistencies in the earlier literature would be explained.

104. *Euxylophora paraensis* Hub.	Amarelo (NP), pau setim, boxwood, buis brésil
Esenbeckia leiocarpa Engl.	Guarantá (NP)
Raputia magnifica Engl.	Arapoca (NP)
R. alba Nees & Mart	Arapoca branca (Freise)
Balfourodendron riedelianum Engl.	Guatambú (NP), guatambu blanco, piquiá marfim (Freise)

Freise (1936) described fifteen cases of dermatitis caused by these satinwood or boxwood substitutes, and two similar cases from the better-known *Fagara flava* (*103*). Most of the eruptions were mild and confined to the finger-webs and other exposed areas, as the sawdust of fresh wood is coarse and does not penetrate under the clothes. Some were more severe, however, with widespread oedema accompanied by general symptoms, usually going on to vesicular dermatitis and often gangrenous ulcers which in three cases proved fatal. Freise confirmed the irritant action of the sawdust on his own skin and on laboratory animals.

Guarantá and amarelo contain the photosensitizer xanthotoxin, and guatambú contains balfourodin and other alkaloids (Hausen, 1970).

105. *Amyris balsamifera* L. (*A. toxifera* Willd.)	Torchwood (NP), amyris, quigua, Venezuelan or W. Indian sandalwood
A. simplicifolia Karst. et spp.	Candil, candlewood, torchwood

Amyris spp. used to be considered one of the main sources of rosewood (Holtzapffel & Royle, 1843; Burn, n.d.), but now provide very little timber (Record & Hess, 1943). Grossmann (1920) claimed that working with *Amyris* and some other rosewoods (see *47*) caused headache, weakness, drowsiness, dyspnoea and sometimes dermatitis. Amyris oil, a substitute for sandalwood oil, was formerly extracted from the wood on a large scale (Record & Mell, 1924). *A. opobalsamum* (not a tree) is the source of Balm of Mecca (Genders, 1972), which was a favourite beauty preparation in the Middle East but could produce alarmingly violent reactions on the face (Montagu, 1717).

106. *Citrus limon* (L.) Burm. f.	Lemonwood, zitronenholz
C. aurantium L. et spp.	Orangewood
C. medica L.	Citron, citronnier

Citronnier used to be popular with French cabinet-makers (Hinckley, 1960), but in Germany the name zitronenholz has been much more widely applied. It seems to have referred mainly to satinwoods (*103*, *108*) with a lemon scent (Gayer, 1928), which were probably responsible for most if not all of the reported cases of zitronenholz dermatitis. Grossmann (1920) doubted whether genuine *Citrus* wood ever caused skin irritation. Janson (1953) refers to one case of wood dermatitis in a paper on citrus fruit dermatitis, but unfortunately gives no details.

Walker (1921) mentions a case of asthma due to orangewood sawdust used for polishing jewels, but no skin tests were done.

FLINDERSIACEAE

107. *Flindersia australis* R.Br.	Crow's ash, moah, Australian teak
F. brayleana F.Muell. *F. pimenteliana* F.Muell.	**Queensland maple,** Australian maple, maple silkwood

Pflanz (1908) reported dermatitis of the face, hands and arms in woodworkers using Australian moah, but he could not reproduce the eruption with sawdust or extracts, either on the patients or himself. Matthes & Schreiber (1914) also investigated the wood, which they differentiated clearly from 'African moah' (see *191*), but they could find no irritants in it either. It is hardly exported now.

MacPherson (1925b) described dermatitis on the face, neck, hands and forearms of a cabinet-maker using Queensland maple, identified as *F. chetawaiana* Bail. None of his workmates had dermatitis, but the dust made some cough and sneeze. No patch tests were made.

Ritchie (1964) examined in detail the wood of fourteen Australian *Flindersia* spp., finding flavonoids, coumarins, furoquinolines, alkaloids, triterpenoids, etc. Their physiological actions were not investigated.

108. *Chloroxylon swietenia* D.C.	**Ceylon satinwood,** E. Indian s., citronnier Ceylan (NP), atlaswood, citronwood

Jones (1904) reported two outbreaks of dermatitis in cabinet-makers working with East Indian satinwood in Glasgow shipbuilding yards. Not all men exposed were affected. The latent period became shorter in successive attacks. Legge (1907) wrote: 'there can be no doubt that East Indian satinwood possesses much more irritant properties than the West Indian variety'. Bidie (1905), however, maintained exactly the opposite. Both types of satinwood had been imported into England since about 1780 (Sheraton, 1803) and there was no attempt at conservation, so that genuine satinwoods were increasingly replaced by substitutes. This may have been one factor producing fluctuations in toxicity; perhaps an element of photosensitivity was another (see *103*).

Auld (1909) found among other substances in the wood the alkaloid chloroxylonine, which Cash (1911) investigated for toxic properties. At first he found it had no effect on his skin, but at the second attempt 10 days later a reaction appeared after 22 days, and at the third attempt after only 40 min. Another subject was sensitized in the same way by repeated tests, but a third remained unaffected. *C. swietenia* also contains possible photosensitizers (see *103*). Hausen (1970) suggests that the sensitization Auld observed might really have been due to these and other contaminants.

PTAEROXYLACEAE

109. *Ptaeroxylon obliquum* (Thunb.) Radlk. (*P. utile* Eck. & Z.)	Sneezewood, paco (NP), nieshout, umtati

The sawdust of umtati has a strong peppery smell and causes violent sneezing, which restricts its commercial use (Boulger, 1908; Chalk *et al.*, 1935). The substance responsible is the hydroxycoumarin nieshoutol (McCabe *et al.*, 1967; Murray & Ballantyne, 1969).

SIMAROUBACEAE

110. *Quassia amara* L.	Quassia, Surinam quassia, bitterwood
Aeschrion excelsa (Sw.) Kuntze (*Picraena excelsa* Lindl., *Picrasma excelsa* Planch.)	Jamaica quassia, bitterwood
Simarouba amara Aubl.	**Simaruba,** marupa (NP), bitterwood
Ailanthus altissima (Mill.) Swingle (*A. glandulosa* Desf.)	Tree of Heaven

Barham (1794) said that workmen disliked working with Jamaica quassia because it 'bittered' their mouths and throats, and Stone (1921) quoted an account of temporary paralysis in a dog's legs after its ulcers had been bathed with a decoction of the wood. These reports hardly seem to justify Moll's (1950) claim that it is the 'most-feared' wood of S. America, for which he quotes no evidence.

Many Simaroubaceae contain bitter principles such as quassine and neoquassine, and both quassias are used medicinally. The traditional method is to stand water overnight in a cup made of quassia wood and drink it in the morning. The bark of *S. amara* and *Khaya senegalensis* (*114*) and the wood of *Picrasma crenata* and (probably) *Q. amara* also contain 2,6-dimethoxybenzoquinone (Polonsky & Lederer, 1959). This was one of the quinones that gave positive patch tests in Heyl's patient with sucupira dermatitis (*82*).

Negroes stripping the root-bark of simarouba (perhaps not *S. amara*) wore protective clothing because the sap would cause *gale* severe enough to prevent them walking for some days (Préfontaine, 1763). There seem to be no recorded reactions to the timber, which is the only one of the family used commercially.

Gardeners pruning the Tree of Heaven may get vesicular eruptions on the face and hands (Cornevin, 1887). It is often planted ornamentally in the U.S.A. but its timber is useless (Record & Hess, 1943).

BURSERACEAE

111. *Aucoumea klaineana* Pierre	**Gaboon,** okoumé (NP), angouma, Gaboon mahogany

Spitzer (1927) reported dermatitis of the exposed skin in a furniture maker working with okoumé. Patch tests were positive on a control subject with eczema and negative on a normal control, but were not done on the patient. Subiza (1958) found that nearly all the workers exposed to a batch of 'okumen carcomido' had conjunctivitis and severe nasal irritation, but few had skin irritation.

One patient with dermatitis seen at St John's gave positive patch tests with gaboon and deal (*203*).

MELIACEAE

112.	*Toona sureni* Merr. et spp. *Cedrela tooni* Roxb.	**Burma cedar,** suren (NP), toon, chomcha, red cedar

The FPRL (1963) receives a few trade reports of skin irritation from this widely used wood.

113.	*Swietenia macrophylla* King (*S. mahagoni*)	Honduras mahogany, Tabasco m., baywood
	S. mahagoni Jacq.	Cuban mahogany, West Indian or Spanish mahogany

Both species are officially called **American mahogany,** acajou amérique (NP).

American mahogany was first imported to Spain before 1600, and to England not long after. Since the eighteenth century it has been prized as a cabinet wood, but with increasing scarcity it has been gradually replaced in Europe by African mahogany (*114*) from the late 19th century onwards (Lamb, 1966; Record & Hess, 1943).

Czimatis & Hagemann (1910) and later authors (see Hausen, 1970) have listed mahogany as toxic without distinguishing between *Swietenia* and *Khaya*. The only satisfactory account of dermatitis certainly due to *Swietenia* sp. is that of Steiner & Schwartz (1944). They described an outbreak of rather mild dermatitis in American shipbuilders working with Tabasco mahogany. Only two of the four men patch-tested with the sawdust reacted positively, but another reacted to an ether extract. The outbreak subsided as the weather became cooler.

Grossmann (1920) thought that American mahogany was harmless, but he had seen dermatitis caused by potassium dichromate used to stain lighter mahogany substitutes. *Swietenia* is botanically so close to *Khaya*, however, that other species besides *S. macrophylla* may well prove to be allergenic.

One St John's patient gave a positive patch test with Honduras mahogany (*207*).

114.	*Khaya ivorensis* A.Chev. (*K. klainei* Pierre) *K. anthotheca* C.DC.	**African mahogany,** acajou d'Afrique (NP), ogwango, krala, acajou blanc, mangona, mbawa, munyama
	K. grandifoliola C.DC.	Big-leaf mahogany, bissilom (NP)
	K. senegalensis A.Juss.	Dry-zone mahogany, bissilom (NP)
	K. nyasica Stapf.	Mhangazi, E. African mahogany

The British Standard name for all *Khaya* spp. is **African mahogany.**

The species are difficult to distinguish, especially after felling. They are often imported mixed, though *K. ivorensis* provided the bulk of the timber until recently (Rendle, 1956). The E. African species have not been reported to cause dermatitis.

Czimatis & Hagemann (1910) included *K. senegalensis* in their list of toxic timbers. There were no case reports referring specifically to *Khaya*, however, until Morgan & Wilkinson (1965) described two outbreaks of dermatitis in a furniture factory, affecting the faces, forearms and hands of men engaged in sanding and other finishing operations. Patch tests were positive with sawdust of *K. anthotheca* only, but *K. euryphylla* is now known to cross-react with it sometimes (Wilkinson, 1971). Two cases sensitive to *K. ivorensis* have since been found, but they did not cross-react with *K. anthotheca* which remains much the commoner sensitizer (Morgan *et al.*, 1968; Wilkinson, 1970). Its principal allergen is anthothecol (Fig. 7), but there may be others.

Mucosal irritation was the main feature of another outbreak described by Wilkinson (1970), but it was not certain that this was due to *Khaya*. Sosman *et al.* (1969) described asthma in a pattern-marker with positive inhalation test and precipitating antibody in the serum to 'mahogany', but as this was in New England it could have been either *Khaya* or *Swietenia*.

Shevlyakov (1969) found that fifteen of sixty-eight Russian furniture workers using 'mahogany from Congo and Ghana' (evidently not *Swietenia mahagoni* as he thought) had dermatitis on the forearms, elbow flexures, groins, face and neck rather than the hands. Patch tests were positive in five of twenty-one polishers tested.

115. *Entandrophragma utile* Sprague — **Utile,** sipo (NP), assié, kalungi, mfumbi
E. cylindricum Sprague — **Sapele,** sapelli (NP), aboudikro

The Forestry Department in Ibadan, Nigeria, receives occasional trade complaints of irritation by utile (Wendorff, 1964). Wilkinson (1971) has seen one case of utile dermatitis in England, and one St John's patient was sensitive to utile and rosewood (*217*), but the wood is extensively used and the incidence of toxic effects probably low.

Brezina (1913) described a scarlatina-like eruption on the arms which followed polishing 'sapeli-mahogany', but no patch tests were made and the identification is none too certain. Two cases sensitive to sapele have been seen at St John's (*214*).

116. *Lovoa klaineana* Pierre ex Sprague (*L. trichilioides* Harms.) — **African walnut,** dibétou (NP), apopo, tigerwood, side

Workers in one Ibadan factory complained of nasal irritation from this wood (Woods, 1964). One man with dermatitis gave positive patch tests with African walnut and mansonia at St John's (*219*).

117. *Guarea cedrata* Pellegr.
G. thompsonii Sprague & Hutch.
G. laurentii De Wild. — **Guarea,** bossé (NP), obodo, Nigerian pearwood, N. cedar, cedar mahogany, bosassa

G. trichilioides L. et spp. — Cramantee (NP), muskwood

All the men in contact with guarea in one factory complained of severe irritation of the nose and throat (MacKenna & Horner, 1954). Trade complaints of skin and nasal irritation are only occasional in Nigeria (Wendorff, 1964; Woods, 1964), but common in England (Orsler, 1969). *G. thompsonii* is said to be more irritating than *G. cedrata* (Farmer, 1972).

The American species (mainly *G. trichilioides*) smell strongly of musk and their powdered bark is emetic and haemostatic (Record & Hess, 1943). They are much less important than the African ones as timber.

118. *Turraeanthus africanus* Pellegr. *T. vignei* Hutch. & J.M.Dalz.	**Avodiré** (NP), apaya, engan, lusamba

A few trade complaints of skin irritation have been reported to the FPRL (1963) and to Hausen (1970). Wilkinson (1971) has seen two cases of avodiré dermatitis with positive patch tests. In Ghana the sawdust is said to make woodcutters spit blood (Irvine, 1961). The wood contains one or more alkaloids (Hausen, 1970).

119. *Dysoxylum muelleri* Benth.	Stavewood (NP), red bean, miva mahogany
D. fraseranum Benth.	Rose mahogany

Cabinet-makers in Australia found that after working with red bean for 4 or 5 days 'they began to suffer from a virulent form of influenza, accompanied by violent fits of vomiting and bleeding at the nose' (Maiden, 1909). The more seasoned the wood was, the worse it became. Aldersley (1925) reported the same effects, but used the wrong botanical name for red bean (see Cleland, 1925). Bisset (1949) vividly described his own similar symptoms, accompanied by painful breathing, headache and anorexia from which he did not fully recover for 4 days, after sawing up the wood to obtain dust for extraction. He found white deposits in the parenchyma cells of *D. muelleri* but not of non-irritant species of *Dysoxylum*, and intended to examine them pharmacologically.

The sawdust of *D. muelleri* and *D. richii* can cause eczema (Maiden, 1909; Nomenclature Générale, 1965). *D. fraseranum* and other species are also toxic (Hausen, 1970).

120. *Sandoricum indicum* Cav. et spp.	**Katon,** kra-thon (NP), sentul, ketjapi, thitto

MacKenna & Horner (1954) described sneezing, epistaxis, tightness of the chest, body-aches, 'marked mental dimness' and bradycardia which affected four men sawing katon, and to a lesser degree other men nearby and the investigating physician. There was no dermatitis. The heartwood contains the triterpene katonic acid (King & Morgan, 1960).

121. *Amoora polystachya* Hook. f. & Jackson *A. cucullata* Roxb. et spp.	**Tasua** (NP), amari, goi

The FPRL (1963) has received a few trade reports of skin and mucosal irritation and general symptoms.

EUPHORBIACEAE

122. *Euphorbia tirucalli* L.	Milk-hedge
E. continifolia L.	Lechero
E. candelabrum Trem.	

Caustic milky sap is characteristic of the Euphorbiaceae, and their timber is so troublesome to fell and work that few species are exported. Dermatitis from the wood of *E. candelabrum* which was used in Somalia for making caskets was described by Lipparoni (1951), and from the sap of other species by Raymond (1936), Blohm (1962) and others. The latex contains a number of tetracyclic triterpenes (euphol, euphorbol, cycloartenol, etc.) and other substances, the physiological actions of which do not seem to have been studied (Ponsinet & Ourisson, 1968).

123. *Hippomane mancinella* L.	Manzanillo (NP), manchineel, beach apple, arvore da morte

The manchineel is one of the most notorious of all irritant trees, and has accumulated an extensive mythology (Bodeau, 1936). Soldiers training in the West Indies during the last war often had bullous dermatitis and severe conjunctivitis after accidental contact with the sap (Satulsky & Wirts, 1943; Grana, 1946). The irritant can be effectively removed from the skin within 30 minutes by soap and water, or more traditionally by plain sea-water as the tree is usually found on or near beaches (Snow & Harley, 1944).

Woodcutters char the bark before cutting the tree down (Barham, 1794; Allen, 1943). The wood is used locally for turnery and cabinet-work, but the sawdust causes rhinitis and cough and some men refuse to work with it (Earle, 1938).

124. *Hura crepitans* L.	**Hura**, assacú (NP), sandbox, possentrie, possumwood, javillo, sablier, ceiba blanca

Possen- and possum-tree are corruptions of poison tree (Record & Mell, 1924).

'The fellers, as they cut them down, are very careful of their eyes; and those that have Cipers [kerchiefs], put it over their faces; for if any of the sap fly into their eyes, they become blind for a month.' (Ligon, 1673). Sandbox sap causes blisters and erysipelas-like eruptions (Pardo-Costello, 1923; Allen, 1943), but the wood is exported and has produced dermatitis in Europe (Hausen, 1970). It was one of the first toxic woods to be chemically investigated. Boussingault & Rivero (1825), working in Bogotà, found a vesicant essential oil in the latex, the vapour from which inflamed their faces severely while it was being boiled down. Richet (1909, 1910) studied anaphylaxis provoked in animals by an albuminoid extract of the latex which he called crepitine, but this was not the irritant principle. The latex contains complex triterpenes like those of *Euphorbia* species (Ponsinet & Ourisson, 1965).

125. *Spirostachys africanus* Sond. (*Excoecaria africana* M.Arg.)	African sandalwood, sandalo africano, tambootie, tomboti
Excoecaria agallocha L.	Geor (NP), aloewood, blind-your-eye

Small boxes and ornaments are made locally from African sandalwood, which is also one source of sandalwood oil. The sawdust irritates skin and mucosae (Codd, 1951; Pardy, 1954), and yokes made of the wood had to be discarded because they inflamed the oxen's necks (Davy, 1929).

The sap of geor blisters woodcutters (Behl & Captain, 1966) and the wood is said to irritate skin and eyes even when dry (Lewin, 1928).

126. *Croton megalocarpus* Hutch.	**Musine**
Sapium ellipticum Pax.	Tomi

Musine has been used in England for flooring. Its fresh wood has an objectionable smell, and the sawdust irritates the nose and throat of some workmen (Anon., 1951).

Several *Sapium* spp. contain vesicant latex, but none produces timber of commercial importance (Standley, 1927; MacMillan, 1957; Irvine, 1961; Lewin, 1962).

BUXACEAE

127.	*Buxus sempervirens* L.	**European boxwood,** Abassian-, Iranian-, Turkey-boxwood
	B. macowani Oliv.	**East London boxwood,** Cape boxwood

Markin (1930) reported asthma with a positive scratch test with box sawdust in a watchmaker who used it to clean gold. Contact dermatitis from the leaves was described by White (1889) and Oppenheim (1913), but the only two cases ascribed to the wood are Nestler's (1929) with very dubious identification, and Nordin's (1947), a very brief account without patch tests. Grossmann (1920) thought mild general symptoms could follow prolonged work with boxwood.

Several unrelated boxwood substitutes are toxic (*104, 183, 195*). The Australian boxthorn mentioned by Cleland (1914) is not *Buxus* but probably *Bursaria spinosa* (Boulger, 1908; Baker, 1919).

ANACARDIACEAE

128.	*Anacardium excelsum* Skeels *A. occidentale* L.	Espavel (NP), cashew, cajú, caracoli, maranon

The shell of the cashew nut from *A. occidentale* is a strong sensitizer (Préfontaine, 1763; Downing & Gurney, 1940; Behl & Captain, 1966), and the wood perhaps also causes dermatitis (Lewin, 1928). The active principle is cardol (Fig. 2). *A. execlsum* produces more important timber, but there are no reports of dermatitis from this.

129.	*Gluta renghas* L. et spp. *Melanorrhoea laccifera* Pierre *M. wallichii* Hook.f. et spp.	Rengas (NP), gluta, rak, son, torotoro, Singapore mahogany
	M. usitata Wall.	Theetsee, Burma lacquer

Rengas is the Malay name also for several species of *Semecarpus* (*139*), *Melanochyla* and *Swintonia*, the common factor being highly irritant sap which rapidly blackens on exposure to the air (Burkill, 1935; Corner, 1952). The attractively figured wood is now little exported because of its toxicity, but it may have been the source of the Borneo rosewood (Gimlette, 1929) and magenta rosewood which caused dermatitis described by Legge (1906, 1907). Men coat their skin with coconut oil before felling the tree and then leave the trunk to dry before cutting it up, but even dry wood in old furniture can produce very irritating dust (Ridley, 1922). The latex contains glutarenghol and thitsiol (Fig. 2) (Majima, 1922; Schwartz *et al.*, 1945).

130. *Mangifera caesia* Jack.	Binjai, wild mango
M. lagenifera Griff.	Lanjut
M. odorata Griff.	Kwini
M. indica L.	Mango

Corner (1952) considered the sap of binjai and lanjut the most poisonous and that of the common mango harmless, but he did not distinguish between irritation and sensitivity. Patients with dermatitis from mango fruit in fact react strongly to the sap, and also cross-react with poison ivy and pentadecylcatechol (Simmons & Bolin, 1921; Keil *et al.*, 1946). *Mangifera* spp. produce no timber for export, but dermatitis from kwini wood has been reported in Malaya (Ridley, 1922). The smoke from a fire of binjai branches caused dermatitis in several people (Landor, 1943).

131. *Spondias mombin* L.	Mopé (NP), mombin, jobo

A Nigerian sawyer developed mild papular dermatitis on exposed skin. After it had subsided he was patch-tested with sawdust from seven woods he had recently worked with. *S. mombin* gave a positive (+ +) reaction, and *Pterygota macrocarpus* and *Terminalia ivorensis* slight (±) reactions. No other Anacardiaceae were available to test for cross-sensitivities (Woods, 1964).

132. *Rhus vernicifera* DC.	Japanese lacquer tree
R. striata Ruiz & Pavon	Manzanillo
R. toxicodendron L. et spp.	Poison ivy, poison oak
Cotinus coggygria Scop. (*Rhus cotinus* L.)	Wig tree, fustic wood, fisetholz

The lacquer of *R. vernicifera* is the most toxic of those produced by the Anacardiaceae, containing urushiol (Fig. 2) (Majima, 1922). It was known to cause dermatitis in the seventh century in China (Toyama, 1918) and in the eighteenth century in England, when cabinet-makers called it the Indian varnish (Bateman, 1819). The wood is not exported but has been used for cabinet-making in Japan (Yoshida, 1884). In North Vietnam shoes made of its cause dermatitis, and when used for firewood it produces smoke with highly irritating particles (Holcik, 1959).

In Venezuela, *R. striata* is called palo hinchon: 'the tree which swells', from the effect on the skin of its wood and sap (Hurtado, 1965, 1968). The North American species of *Rhus* (Dawson, 1954; Kligman, 1957) produce no wood large enough to be useful.

The wood of *Cotinus coggygria* has been known to cause dermatitis and general symptoms when cut for veneers (Hausen, 1970), but it is more often used as the source of the yellow dye fustic.

133. *Metopium brownei* (Jacq.) Urban (*M. toxiferum* Krug. & Urb., *Rhus metopium* L.)	Checham (NP), black poisonwood, doctor's gum

The bark causes blistering and ulcerating dermatitis in those who fell the tree (Pardo-Costello, 1923). The wood is harmless, but is only used locally (Record & Hess, 1943).

134. *Lithraea caustica* Hook. & Arn. Lithi, litre, hualtaco, aroeira
L. brasiliensis March.

'In Chili, there is a very common tree called licti, the shade whereof causes the bodies to swell of those that sleep under it; but more especially the face, so that they cannot see out of their eyes' (Barham, 1794). Later authors confirmed that the sap, and at certain times of year the exudate from the leaves, are highly irritant (Sprague, 1921). When the wood is dry it is said to be harmless and has been used for cabinet-work (Webster, 1911), but some dermatitis occurs even then (Hausen, 1970). See also *136*.

Lima (1953) and Hurtado (1968) studied cross-sensitivity between *Lithraea* spp., *Rhus* spp. and other S. American Anacardiaceae such as *Mauria puberula.*

135. *Schinopsis lorentzii* Engl. Quebracho colorado (NP), red q., san juan, pau mulato
S. balansae Engl.

In Santiago, dermatitis from the leaves, branches or sawdust of this tree is called paaj or mal de quebracho (di Lullo, 1928, 1934). It lasts 5–10 days, and is often accompanied by fever, headache and anorexia. Patch tests with the leaves or an alcoholic extract were positive in nearly half the local people tested in Chile.

The very hard wood (quebracho means axe-breaker) is used more for tanning than timber. White quebracho is an unrelated species of *Aspidosperma* (*179*).

136. *Schinus terebinthifolius* Raddi Aroeira
S. molle L. Pepper-tree

Von Bassewitz (1928) wrote at length about dermatitis, conjunctivitis and mucosal irritation caused by aroeira in Brazil, and the folklore surrounding it. Like Freise (1932), he thought it was probably a species of *Schinus*, but *Astronium* (*137*) and *Lithraea* (*134*) are also called aroeira.

137. *Astronium fraxinifolium* Schott **Gonçalo alves,** urunday (NP), zebrawood, courbaril, locust wood, kingwood, tigerwood, guaribu, roble gateado, aroeira
A. graveolens Jacq. et spp.

In Brazil the sawdust and turpentine-like balsam of this wood and some *Schinus* spp. (*136*) cause an irritable weeping skin eruption, often complicated by erosions like second-degree burns (Freise, 1932). Native forestry workers believe that after the first attack they become immune to it, and Indians are always immune.

The wood often resembles that of *Dalbergia* spp. (*94*) and may be mixed with shipments of them (Record & Mell, 1924; Record & Hess, 1943).

138. *Smodingium argutum* E.Mey. Um-tovane, tovana

Findlay (1963) collected seven cases of dermatitis from the sap of this small tree in S. Africa, some with positive patch tests. It contains at least two unsaturated heptadecylcatechols similar to those of poison ivy (Findlay *et al.*, 1974). The timber is of no importance.

139. *Semecarpus anacardium* L.f.	Indian marking-nut, bhilawa
S. venosa Volk.	Aangot
S. australiensis Engl.	Tar tree
Blepharocarya involucrigera F.Muell.	Bolly gum, rose butternut

The fruits of the marking-nut tree cause dhobie-mark dermatitis (Livingood *et al.*, 1943; Behl & Captain, 1966). 'The wood contains an acrid juice which causes swelling and irritation, and timber-cutters object to felling it; it is not used' (Gamble, 1902). The sensitizers are semecarpol and bhilawanol (Pillay & Siddiqui, 1931). The sap of the tar tree (Flecker, 1945) and the related bolly gum produce dermatitis in Australia (Cilento, 1944; Webb, 1948a). Other skin-sensitizing species are *S. venosa* in Japan (Iseki, 1932) and *S. laxiflora* in Melanesia (Record, 1945).

CELASTRACEAE

140. *Euonymus europaeus* L.	Spindle tree

The bark, leaves and fruit contain a toxic glycoside (Cornevin, 1887; Santavy & Reichstein, 1948), but there is probably little of it in the wood, which used to be a favourite material for cups, casks and especially meat-skewers (Loudon, 1844; Boulger, 1907).

ACERACEAE

141. *Acer saccharum* Marsh. et spp.	**Maple**

Maple-bark disease is a pulmonary hypersensitivity reaction to the fungus *Cryptostroma corticale*, with cough, night sweats and radiographic evidence of infiltration (Emanuel *et al.*, 1966). A similar disease caused by *Alternaria* spores from other types of wood was described by Schlueter *et al.* (1972). No published evidence has been found to support Weber's (1953) listing of maple as a skin irritant.

RHAMNACEAE

142. *Colubrina ferruginosa* Brogn. et spp.	Bois pelé (NP), snakebark, bois serpent, ironwood, sobragy

Vorreiter (1949/1958) lists this locally-used West Indian wood as a cause of vomiting, diarrhoea and cramps, but quotes no source. No other reference to toxic effects has been found.

MALVACEAE

143. *Hibiscus elatus* Sw.	Blue mahoe, majagua

The wood smells peppery when worked and causes sneezing (Stone, 1924; Record & Mell, 1924).

STERCULIACEAE

144. *Nesogordonia papaverifera* Capuron
(*Cistanthera papaverifera* A.Chev.)

Danta, kotibé (NP), epro,
otutu, ovové, aborbora

Some complaints of irritation by this wood are received by the Forestry Dept. in Ibadan (Wendorff, 1964).

145. *Sterculia caribaea* R.Br.
S. oblonga Mast.

Chicha (NP), maho, bois de caca
Yellow sterculia

MacKenna & Horner (1954) include maho among the woods whose 'chief, if not only, effect is on the skin'. Anon. (1957) and Zafiropoulo *et al.* (1968) also list it, but no case reports have been found.

Yellow sterculia has a strong disagreeable odour when fresh (Farmer, 1972), a characteristic of the genus reflected in the names sterculia and bois de caca. Karaya and other gums from *Sterculia* spp. have been reported to cause asthma, rhinitis and urticaria (Feinberg & Schoenkerman, 1940; Bowen, 1939).

146. *Triplochiton scleroxylon* K. Schum.

Obeche (NP), **wawa,** abachi,
African whitewood, samba

Bridge (1937) reported a case of asthma due to the sawdust, skin tests being positive with obeche and negative with other woods. Oehling (1963) described three cases of obeche asthma (one also sensitive to limba, *159*) with positive intradermal, inhalation and Prausnitz-Küstner tests. He described contact urticaria, which could be reproduced by rubbing the skin with a slip of obeche but not with other woods, and also found positive ID tests with it in many asthmatic subjects who had been exposed to obeche sawdust but did not react to it clinically.

The FPRL (1963) has five trade reports of asthma and general symptoms, but none of skin eruptions. Wilkinson (1971) has seen a case of dermatitis from a lavatory seat made of obeche, with positive patch test. Hausen (1970) found no alkaloids or quinones in the wood.

147. *Mansonia altissima* A.Chev.

Mansonia, bété (NP), aprono,
ofun, koul, Nigerian walnut

Mansonia was introduced from Nigeria into the world market in the thirties, but its irritant properties soon became notorious (Bridge, 1935). Nearly all men exposed to the fine dust through sawing and especially sandpapering complained of sneezing and sore throat, and many had nosebleeds and headache. Some also developed dermatitis on exposed skin with positive patch tests (Horner & Wigley, 1936; Bourne, 1956). Salamone *et al.* (1969) also found skin irritation with a follicular eruption in a minority of their patients, but patch tests were negative. Mucosal irritation affected many more. Despite exhaust ventilation and other precautions mansonia is still considered a troublesome wood to work (Orsler, 1969) and this restricts its use to some extent (Rendle, 1969; Farmer, 1972). Four cases of mansonia dermatitis have been seen at St John's (*208*).

Mascré & Paris (1939) found in the bark a cardiac glycoside which they called mansonine. Several other glycosides related to strophanthin have since been found in the bark and heartwood (Frèrejacque, 1951; Sandermann & Dietrichs, 1959). Sandermann & Dietrichs also isolated

205. *Laurel, Indian (159)*

Case 24 (M85588). A 46-year-old man worked as a veneer preparer. He developed an extensive dermatitis of the face, head and neck, which spread to the trunk and limbs. His eyelids had been swollen. He gave positive patch tests to Indian laurel, teak, Honduras mahogany, jacaranda rosewood and primula; doubtful (±) reactions to ash, pine and elm; and was negative to makoré, African walnut, and to wood and coal tars. He was positive to R- and S-dalbergione but negative to lapachol, pinosylvin and anthothecol.

206. *Louro (44)*
Five men reacted to louro when using it as a veneer in a furniture factory. Two were definite and three were doubtful reactions. Seventeen other men with dermatitis who were tested gave negative reactions.

Case 25 (K348). A 40-year-old wood machinist had a recent history of dermatitis of the face and arms. Patch tests were positive to louro, but negative to ayan and afrormosia.

Case 26 (K1552). A 52-year-old furniture worker had psoriasis and angioma serpiginosum. For 2 weeks he had a dry scaly dermatitis of the face and neck. Patch tests were positive to louro, but negative to ayan and afrormosia.

Case 27. A 30-year-old furniture worker gave a 2 month history of dermatitis of his forehead and the dorsa of his hands. Patch tests were weakly positive (±) to louro and negative to ayan and afrormosia.

Case 11. See para. *199* Ayan.

Case 13. See para. *199* Ayan.

207. *Mahogany, Honduras (113)*

Case 24 (M85588). See para. *205* Laurel.

208. *Mansonia (147)*
Four patients were positive to mansonia, which was imported in large quantities between 1945 and 1955. One was a female cleaner who only swept up sawdust and had dermatitis of the feet, while the three men were a joiner, a cabinet-maker and a sawmill grinder.

Case 28 (F10722). A 63-year-old joiner gave a 3 month history of dermatitis of the face, eyelids, scalp, arms and axillae. Patch tests were positive to mansonia and to teak.

Case 29 (C12170). A 58-year-old grinder in a sawmill had a long-standing leg ulcer and a more recent dermatitis of the face. Patch tests were positive to mansonia, sapele and teak (±), but negative to obeche, ebony, agba, abura, mahogany, Japanese oak, blackwood and walnut.

Case 30 (C12714). A 58-year-old female cleaner in a school had a dermatitis of the hands which was thought to be due to alkali and detergents. She also had dermatitis of the feet which she said was due to sweeping up sawdust in the carpentry room at the school. Patch tests were positive to mansonia, but negative to teak, agba, abura, obeche, ebony, walnut, mahogany, sapele and boxwood.

Case 31 (C13590). A 54-year-old cabinet-maker had dermatitis of his face and wrists. Patch tests were positive to mansonia and rosewood, but negative to makoré, ash, mahogany and teak.

209. *Oak (24)*

Case 32 (H9198). A 48-year-old woodworker had extensive atopic dermatitis. Patch tests were positive to teak and oak.

Case 33 (H9647). A 40-year-old housewife had a dermatitis which was thought to be due to the wearing of textiles. Patch tests were positive to formalin and to oak.

Case 20 (A9405). See para. *202* Cedar.

210. *Pine, Deal (7)*

Case 34 (M33170). A 33-year-old carpenter worked mainly on the erection of display stands for exhibitions. He handled many primary irritants. He gave a 6 month history of dermatitis of both palms. Patch tests were positive to Norwegian red deal but negative to teak and utile.

Case 35 (M78794). A 43-year-old carpenter had a 10 year history of recurrent dermatitis of the face, eyelids, ears, neck, hands and scrotum. Patch tests were positive to teak, pine, phenol formaldehyde and lapachol, but negative to dalbergione, pinosylvin, anthothecol, and to wood and coal tars.

Case 36 (M93323). A 16-year-old carpenter apprentice had a 1 year history of dermatitis of the palms and fingers, face, eyelids, and around the mouth. It cleared when he was away from work. Patch tests were positive to deal (+ +), pinosylvin, anthothecol, colophony, formalin and turpentine, but negative to dalbergione, pinomonosylvin monomethyl ether, wood and coal tars.

Case 21 (93521). See para. *203* Gaboon.

Case 37 (M8733). A 55-year-old cabinet maker had an oedematous dermatitis for a month of his face and elbow flexures. Patch tests were positive to Portuguese pine and hardboard, but negative to gaboon, mahogany, teak and oak.

Case 17. See para. *200* Birch.

211. *Pine, Parana (3)*

Case 38 (M91544). A 64-year-old carpenter had had attacks of dermatitis over a period of 5 years, affecting his eyelids, hands and legs. He had used an epoxy resin adhesive on some wood. Patch tests were positive to ramin, parana pine and teak, as well as to epoxy resin and to wood and coal tars. It was not possible to be certain that his samples of wood were not contaminated with cured resin but we think this unlikely.

212. *Ramin (157)*

Case 38 (M91544). See above Parana Pine.

213. *Rosewood (94)*
Eight patients were sensitive to rosewood (*Dalbergia* spp.), seven men and one woman. Their ages ranged from 46 to 60 years at the time of presentation. Their occupations were musical instrument maker, wood machinist (2), cabinet-maker (2), timber yard foreman, veneer preparer and housewife. Three had involvement of the genitalia or groins, and one had cheilitis from holding wood in his mouth.

Case 39 (F14226). A 43-year-old man who was a clarinet player, worked on making musical instruments using a lot of rosewood. For 15 years he had had recurrent attacks of dermatitis of his eyelids, lips, face, neck, elbow flexure and of the genitalia. Patch tests were positive to rosewood, but negative to primula leaf, colophony and formalin.

Case 40 (E10462). A 59-year-old cabinet maker had a 9 week history of dermatitis of his neck, scalp, face, arms and chest. Patch tests were positive to wood dust from his workshop, rosewood and utile, but negative to walnut, ramin, oak, pine and mahogany.

Case 41 (G11982). A 59-year-old wood machinist gave a week's history of dermatitis of his face, neck and wrists. Patch tests were positive to rosewood and teak, but he was negative to afrormosia, mahogany and meranti.

Case 31 (C13590). See para. *208* Mansonia.

Case 42 (M26610). A 58-year-old timber foreman gave a 2 year history of dermatitis of his face, eyelids, hands, arms and groins. He had noted that holding a piece of rosewood in his lips resulted in acute dermatitis. Patch tests were positive to rosewood and teak, wood tars, coal tars, balsam of Peru, chromate and paraphenylenediamine. He was negative to primula, colophony and turpentine. Tests with wood allergens gave positive reactions to pinosylvine, anthothecol, R- and S-dalbergione, and lapachol. This man was so sensitive to rosewood that some of these reactions may have been false positives.

Case 43 (M45787). A 60-year-old housewife had worked with rosewood. In the past year she had had three attacks of dermatitis of her legs, arms and cubital fossae, accompanied by sneezing. Patch tests were positive to rosewood, but negative to beech, primula, wood and coal tars, colophony, turpentine and balsam of Peru.

Case 44 (H11491). A 49-year-old wood machinist had attacks of dermatitis of the face, eyelids, axillae, elbow flexures and genitalia. Patch tests were positive to rosewood but negative to makoré and teak.

Case 24 (M85588). See para. *205* Laurel.

The patch test results show that all eight patients reacted to rosewood dust. Additional woods reacting in these patients were utile (1), Honduras mahogany (1), Indian laurel (1), mansonia (1) and teak (2). Only two of the patients could be tested to the wood allergens; both were positive to dalbergione (0·1% in petrolatum), the allergen in rosewood. The patient (Case

45) who also reacted to teak was positive to lapachol, pinosylvin and anthothecol, but these reactions could have been false positives. They were not repeated later. However, *Dalbergia nigra* (Brazilian rosewood, jacaranda) belongs to the family *Leguminosae* and is not related to *Jacaranda* spp. which are known to contain lapachol. There is, therefore, no evidence of cross-reaction between woods in our patients, and the multiple sensitivities are likely to be separately acquired allergies.

Not all the cases were tested to a standard series of contact allergens, but three patients were negative to primula, colophony and turpentine. One was positive and one negative to wood and coal tars and balsam of Peru.

A possible allergenic relationship between rosewood and primula was suggested by Hjorth (1965) and by Wilkinson (personal communication, 1970) who found some rosewood-sensitive patients positive to primula.

214. *Sapele (115)*

Case 45 (M34971). A 57-year-old wood machinist had had three attacks of dermatitis and swelling of his eyelids in a month. It spread to involve his face, neck, scalp, elbow flexures, groins, popliteal spaces and feet. Patch tests were weakly positive to sapele but negative to the European standard series, to obeche and African mahogany.

Case 29 (C12170). See para. *208* Mansonia.

215. *Spruce (4)*

Case 46 (86216). A 30-year-old musical instrument maker had a previous history of infantile eczema and of dermatitis from a watch. For 9 months he had dermatitis of the back of his hand and the skin of the thumb and index finger web. Patch tests were positive to spruce and to casein glue.

216. *Teak (188)*

Case 47 (E5972). A 47-year-old wood machinist had a 10 year history of dermatitis of the face, neck and eyelids. Patch tests were positive to teak but negative to abura, boxwood, obeche, mansonia, beech and walnut.

Case 48 (E3965). A 52-year-old joiner had recurrent dermatitis of the face, eyelids, arms and genitalia for 3 years. A patch test was positive to teak dust.

Case 49 (D516). A 51-year-old wood machinist had had attacks of dermatitis of the face, neck and dorsa of his hands over 4 years. Patch tests were positive to teak dust and to a petroleum ether extract of it but negative to alcohol and aqueous extracts. He was also negative to oak, walnut, mahogany, ramin, obeche, agba and rosewood.

Case 50 (H2735). A 57-year-old joinery labourer with dermatitis had a positive patch test to teak dust and to solvent extracts, but was negative to mansonia and iroko.

Case 51 (B12350). A 53-year-old carpenter had a 6 week history of dermatitis of the face, forearm and scrotum. Patch tests were positive to teak dust but not to solvent extracts of it.

Case 52 (G11982). A joiner gave a history of dermatitis of the eyelids, face, beard area, wrists and hands. Patch tests were positive to teak dust and a petroleum ether extract but negative to alcoholic and aqueous extracts.

Case 53 (G5859). A 47-year-old wood machinist had a 2 month history of dermatitis of the wrists, palms and dorsa of the hands. A patch test with teak dust was strongly positive.

Case 54 (G3498). A joiner stated that he had had dermatitis from teak some years before a recent relapse. Patch tests were positive to teak dust but negative to obeche, beech, pine and iroko.

Case 55 (G3099). A 55-year-old musical instrument maker had a dermatitis of the face, ears, and penis for 6 years. A patch test to teak dust was positive.

Case 56 (G1620). A 58-year-old wood machinist first developed dermatitis from teak 25 years before he presented with an acute attack affecting the face, eyes, hands and arms. A patch test was positive to teak, but negative to abura, sapele, oak and utile.

Case 57 (F5836). A 53-year-old joiner gave a 7 year history of dermatitis of the eyelids, face, beard area, wrists and hands. Patch tests were positive to teak dust and a petroleum ether extract but negative to alcoholic and aqueous extracts.

Case 58 (A7383). A 70-year-old joiner stated that he had had dermatitis from teak 33 years before a recent relapse. Patch tests were positive to teak dust but negative to obeche, beech, pine and iroko.

Case 59 (A3024). A 25-year-old carpenter had five attacks in a month of swelling of his eyelids. Patch tests were positive to teak dust but negative to spruce, pine and obeche.

Case 60 (A3323). A cabinet-maker had a 10 week history of dermatitis of his right forearm, face and ankles. Patch tests were positive to teak but negative to mahogany, pine and cedar.

Case 61 (A4683). A 24-year-old carpenter had a 3 month history of dermatitis of the knee and elbow flexures, later spreading to the hands. Patch tests were positive to teak but negative to oak, spruce and keruing.

Case 62 (C12170). A 58-year-old sawmill grinder had a chronic leg ulcer and a more recent dermatitis of the face. Patch tests were weakly positive to teak dust, positive to mansonia and sapele, but negative to obeche, ebony, agba, abura, mahogany, Japanese oak, blackwood and walnut.

Case 63 (M35286). A 50-year-old butcher had an attack of dermatitis of the hands at the age of 34 years and a recurrence at the age of 49 years. He presented with a recent thickened eczema of the palms. During his work he handled sawdust and did woodwork as a hobby. Patch tests were positive to teak, sawdust from the butcher's shop, balsam of Peru, wool alcohols and tetramethylthiuram disulphide.

Case 64 (M34971). A 57-year-old wood machinist over a period of a month had three attacks of swelling of the eyelids. It later spread to the face, scalp, neck, elbow flexures, groins, popliteal spaces and feet. Patch tests were positive to teak (possibly irritant) and sapele (±), but negative to balsam of Peru, colophony, wood tars, African mahogany and obeche.

Case 65 (M34389). A 36-year-old cabinet maker had a 10 week history of dermatitis of his right forearm, face and ankles. Patch tests were positive to teak but negative to mahogany, pine and cedar.

Case 66 (M32774). A 20-year-old carpenter complained of dermatitis of his neck and shoulders over a period of 8 years. Patch tests were positive to teak dust and weakly (±) to solvent extracts of it, but negative to deal, mahogany and oak.

Case 67 (M33605). A 44-year-old carpenter gave a 1 year history of dermatitis of his hands, wrists, fingers and eyelids. A patch test was strongly positive to teak.

Case 68 (M69261). A 59-year-old wood machinist first had dermatitis from teak some 40 years ago and again 10 years ago. One year ago he had handled teak again with a further recurrence on the hands, arms and legs. He had used a lanolin-containing barrier cream which made the dermatitis worse. Patch tests were positive to teak, wool alcohols, wood and coal tars and Vioform, but negative to lapachol, afrormosia, ash, deal and oak.

Case 69 (M18043). A 63-year-old wood machinist had had two attacks of dermatitis from teak 12 and 6 years ago. One year ago he developed a dermatitis on his legs, arms and face after using teak again. He had a chronic stasis dermatitis. Patch tests were strongly positive to teak and lapachol, and to wood and coal tars.

Case 70 (G1077). A 55-year-old engineer developed a dermatitis of 'seborrhoeic' pattern on the chest, in the groins, axillae and cubital flexures. A patch test was positive to teak dust.

Case 71 (M38417). A 57-year-old carpenter had a 2 week history of dermatitis of the arms, face and genitalia soon after he started working with teak. Patch tests were positive to teak, but negative to oak, mahogany, walnut, deal and pine, as well as to colophony, turpentine and balsam of Peru.

Case 72 (M61466). A 61-year-old painter had been rubbing down some teak wood with sandpaper. Within 4 h his eyelids were swollen and he later developed dermatitis of the face, hands and forearms. Patch tests were positive to teak dust, solvent extracts of it and lapachol and to anthothecol; he was negative to pinosylvine, dalbergione, turpentine and balsam of Peru.

Case 73 (M60100). A 41-year-old wood machinist had had dermatitis of his face, scalp and fingers some 20 years earlier. One year ago he developed a dermatitis of the arms, body, legs, feet, axillae and genitalia. Patch tests were positive to a chipboard, colophony, teak (±) and wood tars (±), but negative to walnut, sapele, lapachol, anthothecol, dalbergione, pinosylvine, formalin and coal tars.

Case 74 (M53957). A 53-year-old carpenter had a month's history of dermatitis of the face and scalp which spread to the arms and thighs. Patch tests were strongly positive to teak dust and to lapachol, but negative to keruing, dalbergione, anthothecol, pinosylvine and wood and coal tars.

Case 75 (92067). A 40-year-old joiner had an 18 month history of dermatitis of the face, chin and eyebrows which he said was aggravated by wood dust. Patch tests were positive to teak but negative to oak, Japanese oak, mahogany and abura.

Case 76 (H9462). A 61-year-old man had had recurrent attacks of dermatitis over 30 years. Patch tests were positive to teak dust and to solvent extracts of it.

Case 77 (H9198). A 48-year-old woodworker had a history of 'atopic dermatitis'. Patch tests were positive to teak and oak.

Case 78 (K1321). A 42-year-old labourer in a wood mill gave a 3 month history of dermatitis of the face, arms, chest and thighs. Patch tests were positive to Burma teak dust and to an ether extract but negative to a chloroform extract of it. Tests were also negative to oak, mahogany, rosewood and obeche.

Case 79 (K2016). A 59-year-old wood machinist had a 3 month history of dermatitis of the hands. Patch tests were positive to teak but negative to sapele and afrormosia.

Case 80 (K9513). A 28-year-old carpenter, making kitchen cabinets and other fittings, had a dermatitis of the face, groins and hands with a lot of oedema. Patch tests were positive to teak dust and to solvent extracts of it.

Case 1 (H11171). See para. *197* Afrormosia.

Case 16 (K11888). See para. *199* Ayan.

Case 17 (M66849). See para. *200* Birch.

Case 18 (G3498). See para. *201* Blackwood.

Case 20 (A9405). See para. *202* Cedar.

Case 22 (A3323). See para. *204* Iroko.

Case 24 (M85588). See para. *205* Laurel.

Case 28 (F10722). See para. *208* Mansonia.

Case 29 (C12170). See para. *208* Mansonia.

Case 32 (H9198). See para. *209* Oak.

Case 35 (M78794). See para. *210* Pine.

Case 38 (M91544). See para. *211* Parana Pine.

Case 41 (G11982). See para. *213* Rosewood.

Case 42 (M26610). See para. *213* Rosewood.

Case 82 (M79021). See para. *219* Walnut.

217. *Utile (115)*

Case 43 (E10462). See para. *213* Rosewood.

218. *Walnut, African (116)*

Case 81 (H8065). A 40-year-old man worked in a wood machining factory. One month after starting he developed a rash on all exposed areas; it improved on stopping work but recurred on returning. Patch tests were positive to African walnut, but negative to African mahogany, red beech and American beech.

219. *Walnut (21)*

Case 82 (M79021). A 43-year-old cabinet-maker gave an 8 month history of an extensive dermatitis of the hands, arms and sides of the neck. He had been receiving treatment for 3 years with systemic steroids for an illness considered to be polyarteritis nodosa with pulmonary involvement.

He used veneers of walnut, avodiré and teak. Scratch tests were negative. Patch tests were positive to paraphenylenediamine, primula, mercaptobenzthiazole, vioform, wood and coal tars, walnut, teak and avodiré. Later patch tests were positive to his own walnut, teak and avodiré veneers, as well as to lapachol and anthothecol. The veneers were identified by the Forest Products Research Laboratory as walnut (*Juglans regia*), teak (*Tectona grandis*) and avodiré *Turraeanthus africanus*). He changed his occupation and made a complete recovery from the dermatitis.

220. *Avodiré (118)*

Case 82 (M79021). See para. 219 Walnut.

PRINCIPAL TOXIC TIMBERS

Trade name	Botanical name	Dermatitis, Mucosal irritation, Asthma, General symptoms	Active substances	Para.
Arbor vitae	*Thuja standishii*	M A	Tropolones	13
Ayan	*Distemonanthus benthamianus*	D	Oxyayanins	70
Blackwood, African	*Dalbergia melanoxylon*	D	Dalbergiones	94
Boxwood, Knysna	*Gonioma kamassi*	M A G	Quebrachamine ?	183
Cedar, Western red	*Thuja plicata*	(D) M A	Tropolones	13
Cocobolo	*Dalbergia retusa* et spp.	D	Dalbergiones	94
Cocus	*Brya ebenus*	D	Quinones ?	91
Dahoma	*Piptadeniastrum africanum*	M	?	62
Ebony	*Diospyros* spp.	D M	Quinones	173
Guarea	*Guarea thompsonii* et spp.	M	?	117
Ipé (lapacho)	*Tabebuia ipe* et spp.	D M G	Desoxylapachol	190
Iroko	*Chlorophora excelsa*	D (M A)	Stilbene	32
Katon	*Sandoricum indicum*	M G	?	120
Mahogany, African	*Khaya ivorensis* et spp.	D (M)	Anthothecol	114
Mahogany, American	*Swietenia macrophylla* et spp.	D	?	113
Makoré	*Tieghemella heckelii*	D M	Saponin	170
Mansonia	*Mansonia altissima*	(D) M G	Mansonones (quinones) Glycosides	147
Obeche	*Triplochiton scleroxylon*	(D) M A	?	146
Opepe	*Nauclea trillesii*	D M	?	195
Peroba rosa	*Aspidosperma peroba*	D M G	Alkaloids	179
Peroba, white	*Paratecoma peroba*	D M	Desoxylapachol ?	191
Ramin	*Gonystylus bancanus*	D	?	157
Rosewoods	*Dalbergia* spp., *Machaerium* spp.	D	Dalbergiones	94
Satinwood, Ceylon	*Chloroxylon swietenia*	D	Alkaloid ? Furocoumarins ?	108
Satinwood, W. Indian (and African)	*Fagara flava* et spp.	D	Alkaloid ? Furocoumarins ?	103
Sequoia	*Sequoia sempervirens*	M G	?	10
Stavewood	*Dysoxylum muelleri*	M G	?	119
Teak	*Tectona grandis*	D	Desoxylapachol	188
Liverworts and lichens on bark	*Frullania*, etc.	D	Sesquiterpene lactones	24

U.K. HARDWOOD IMPORTS (1969)

Separate figures are available only for the species listed (Davis, 1970). Softwoods are imported in much larger quantities but no specific figures are given for toxic ones like *Thuja* spp.

	Thousands of cubic feet	
	Lumber	Logs
Beech	6172	Unspec.
Keruing	2788	Unspec.
Ramin	2211	Unspec.
Utile	2163	1149
Birch	1942	Unspec.
Oak	1021	Unspec.
Obeche	923	1236
Mahogany	646	2088
Teak	495	Unspec.
Okoumé	Unspec.	134
Abura	Unspec.	862
Poplar	Unspec.	382
Unspecified	8271	4543

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Acheson, E.D. (1967) Hodgkin's disease in woodworkers. *Lancet*, **ii**, 988.

Acheson, E.D., Cowdell, R.H., Hadfield, E. & Macbeth, R.G. (1968) Nasal cancer in woodworkers in the furniture industry. *British Medical Journal*, **ii**, 587.

Aldersley, R.B. (1925) Sensitiveness to proteins of wood. *Medical Journal of Australia*, **2**, 25.

Alemany-Vall, R. (1958) Rhinitis and dermatitis caused by exotic woods. In: *Occupational Allergy*, p. 273, Stenfert-Kroese. Leiden.

Allen, P.H. (1943) Poisonous and injurious plants of Panama. *American Journal of Tropical Medicine* (suppl.), **23**, 1.

Altona, T. (1924) Djati en Hindoes. *Tectona*, **17**, 865.

Anderson, A.B. & Zavarin, E. (1955) *Journal of the Institute of Wood Science*, **15**, 3.

Anon. (1906) Poisonous Wood. *Timber Trades Journal*, **60**, 860.

Anon. (1923) Results of the examination of Nigerian timbers. *Bulletin of the Imperial Institute*, **21**, 444.

Anon. (1933) Erkrankungen bei Benutzung von Cocobolo-Blockflöten. *Collegium musicum (Bärenreiter)*, **1**, 13.

Anon. (1955) Douka. *Bois et Forêts des Tropiques*, **42**, 37.

Anon. (1957) Wood dust and the woodworker. *Timber Technology*, **65**, 205.

Arnold, H.L. (1942) Dermatitis due to the blossom of *Grevillea banksii. Archives of Dermatology and Syphilology*, **45**, 1037.

Aronow, L. & Kerdel-Vegas, F. (1965) Seleno-cystathionine, a pharmacologically active factor in the seeds of *Lecythis ollaria. Nature*, **205**, 1185.

Asakawa, Y., Benezra, C., Ducombs, G., Foussereau, J., Muller, J.C. & Ourisson, G. (1974) Cross-sensitization between *Frullania* and *Laurus nobilis. Archives of Dermatology*, **110**, 957.

Aston, B.C. (1923) The poisonous, suspected and medicinal plants of New Zealand. *New Zealand Journal of Agriculture*, **26**, 78 & 149.

Auld, S.J.M. (1909) An examination of irritant woods. 1. Chloroxylonine from E. Indian Satinwood. *Journal of the Chemical Society*, **95**, 964.

Auld, S.J.M. & Pickles, S.S. (1912) The constituents of W. Indian Satinwood. *Journal of the Chemical Society*, **101**, 1052.

Avila, R. (1972) Some aspects of suberosis. *Les Bronches (Paris)*, **22**, 121.

Bahn, K. (1928) Beitrag zur Frage der Allergie bei Holzsägereiarbeitern. *Klinische Wochenschrift*, **7**, 1963.

Baker, R.T. (1919) *The Hardwoods of Australia.* Govt. State N.S.W., Australia.

Barham, H. (1794) *Hortus americanus.* Kingston, Jamaica.

Barnett, G.P. (1948) *Report of the Chief Inspector of Factories*, p. 94. HMSO, London.

Barniske, R. (1957) Dermatitis bullosa ausgelöst durch den Saft grüner Walnussfruchtschalen (*Juglans regia*). *Dermatologische Wochenschrift*, **135**, 189.

Barroso, C. (1947) Dermatite eczematoso de tipo dermocontacto provocada pela serragem de caviúna (*Dalbergia nigra*). *O Hospital (Rio)*, **31**, 291.

Bassewitz, E. von (1928) Die südbrasilianische Aroeirakrankheit. *Archiv für Schiffs- und Tropen-hygiene*, **32**, 494.

Bateman, T. (1819) *A Practical Synopsis of Cutaneous Diseases*, 5th edn, p. 257. Longman, London.

Baumer, M. (1955) Que faut-il croire de la toxicité de certains bois tropicaux? *Bois et Forêts des Tropiques*, **43**, 27.

Behl, P.N. & Captain, R.M. (1966) *Skin-irritant and Sensitizing Plants found in India.* Behl, New Delhi.

Behrbohm, P. (1957) Allergisches Kontakt-Ekzem durch chromathaltige Holzschutzmittel. *Berufsdermatosen*, **5**, 271.

Berlin, C. (1930) Feigenbaumdermatitis. *Dermatologische Wochenschrift*, **90**, 733.

Bernhard-Smith, A. (1923) *Poisonous Plants of all Countries*, 2nd edn. Baillière Tindall, London.

Bevan, C.W.L. (1958) Wood extractives. *West African Journal of Biological Chemistry*, **2**, 36.

Bidie, G. (1905) Satinwoods and dermatitis. *British Medical Journal*, **i**, 74.

Bisset, I.J.W. (1949) Irritant properties of Miva mahogany. *Forest Products News Letter*, no. 178, p. 3.

Black, A., Evans, J.C., Hadfield, E.H., Macbeth, R.G., Morgan, A., & Walsh, M. (1974) Impairment of nasal mucociliary clearance in woodworkers in the furniture industry. *British Journal of Industrial Medicine*, **31**, 10.

BLEUMINK, E., MITCHELL, J.C. & NATER, J.P. (1973) Allergic contact dermatitis from cedar wood (*Thuja plicata*). *British Journal of Dermatology*, **88**, 499.

BLOHM, H. (1962) *Poisonous Plants of Venezuela*. Wissenschaftliche, Stuttgart.

BODEAU (1936) A propos de quelques accidents dus au mancenillier. *Archives de Médecine et Pharmacie Navales*, **126**, 122.

BOLUS, M. (1939) Dermatitis venenata due to Ginkgo berries. *Archives of Dermatology and Syphilology*, **39**, 530.

BOULGER, G.S. (1906/7) *Familiar Trees*. Cassell, London.

BOULGER, G.S. (1908) *Wood*, 2nd edn. Arnold, London.

BOURNE, L.B. (1956) Dermatitis from Mansonia wood. *British Journal of Industrial Medicine*, **13**, 55.

BOUSSINGAULT & RIVERO (1825) Mémoire sur le lait vénéneux de l'hura crepitans. *Annales de chimie et de physique*, **28**, 430.

BOWEN, R. (1939) Karaya gum as a cause of urticaria. *Archives of Dermatology and Syphilology*, **39**, 506.

BREZINA, E. (1921) *Internationale Ubersicht über Gewerbekrankheiten über des Jahr 1913* (Ed. by L.Teleky and E.Brezina). Springer, Berlin.

BRIDGE, J.C. (1935, 1937) *Reports of the Chief Inspector of Factories and Workshops*, London.

BRIGGS, L.H. (1946) Plant products of New Zealand. *Journal of the Proceedings of the Royal Society of New South Wales*, **80**, 151.

BRISSEMORE & COMBES (1905) Sur le juglon. *Comptes rendus de l'Académie des Sciences*, **141**, 838.

BROERS, H. (1926) Dermatitis venenata. In: *Die Schädigungen der Haut durch Beruf und gewerbliche Arbeit* (Ed. by K.Ullmann, J.H.Rille and M.Oppenheim). Voss, Leipzig.

BRÜGEL, S. & PERUTZ, A. (1927) Uber Klinik und Pathogene der Erlenholzdermatitis. *Archiv für Dermatologie und Syphilis*, **153**, 661.

BUHR, A.J. (1960) The thorn in the flesh. *Lancet*, **i**, 309.

BULLETIN (1935) Bulletin de l'Union internationale des Ouvriers du Bois 1931–2. Les Maladies professionnelles dans l'Industrie du Bois. *Annales d'Hygiène publique*, **13**, 139.

BURGESS, J.F. & USHER, B. (1930) On hypersensitiveness to quinine. *Canadian Medical Association Journal*, **23**, 45.

BURKILL, I.H. (1935) *A Dictionary of the Economic Products of the Malay Peninsula*. Crown Agents, London.

BURN, R.S. (n.d.) *The New Guide to Carpentry*. Fullarton, London. (c. 1870).

BURNETT, A.R. & THOMSON, R.H. (1967) The Quinonoid Constituents of *Tabebuia avellanedae* (Bignoniaceae). *Journal of the Chemical Society* (C), 2100.

BURNETT, A.R. & THOMSON, R.H. (1968) Extractives from *Tabebuia chrysantha* Nichols. and other Bignoniaceae. *Journal of the Chemical Society* (C), 850.

BUSCHKE, A. & JOSEPH, A. (1927) Ueber Hautentzündung hervorgerufen durch Makassarholz. *Deutsche medizinische Wochenschrift*, **53**, 1641.

CALNAN, C.D. (1972) Dermatitis from Cedar wood pencils. *Transactions of the St John's Hospital Dermatological Society*, **58**, 43.

CALNAN, C.D. & SHUSTER, S. (1963) Reactions to ammonium persulfate. *Archives of Dermatology*, **88**, 812.

CANCELLA, L.DE C. (1955) On a special kind of pneumoconiosis: the suberosis. *A Medicina contemporânae (Lisbon)*, **73**, 235.

CANCELLA, L.DE C. (1963) Suberosis: a pneumoconiosis due to cork dust—the present stage of the problem. *Industrial Medicine and Surgery*, **32**, 435.

CASH, J.T. (1911) The dermatitis produced by E. Indian satinwood (*Chloroxylon swietenia*). *British Medical Journal*, **ii**, 784.

CHALK, L., CHATTAWAY, M.M., BURTT DAVY, J., LAUGHTON, F.S. & SCOTT, M.H. (1935) *Fifteen S. African High Forest Timber Trees*. Clarendon Press, Oxford.

CHAMPION, R.H. (1965) Wood-cutter's disease: contact sensitivity to lichens. *British Journal of Dermatology*, **77**, 285.

CHAMPION, R.H. (1971) Atopic sensitivity to algae and lichens. *British Journal of Dermatology*, **85**, 551.

CHAN-YEUNG, M., BARTON, G.M., MCLEAN, L. & GRZYBOWSKI, S. (1971) Bronchial reactions to Western Red Cedar (*Thuja plicata*). *Canadian Medical Association Journal*, **105**, 56.

CHITTENDEN, F.J. (1931) Plants causing skin irritation. *The Gardener's Chronicle*. 24.10.31, pp. 332–334, 353.

CHOW, Y.L. & QUON, H.H. (1968) Chemical constituents of the heartwood of *Mesua ferrea*. *Phytochemistry*, **7**, 1871.

CILENTO, SIR R. (1944) *Some poisonous plants, sea and land animals of Australia and New Guinea*. Smith and Paterson, Brisbane.

CLELAND, J.B. (1914) Plants, including fungi, poisonous or otherwise injurious to man in Australia. *Australasian Medical Gazette*, **35**, 541 & 569.

Cleland, J.B. (1925) Plants, including fungi, poisonous or otherwise injurious to man in Australia. *Medical Journal of Australia*, **ii**, 443.

Cleland, J.B. (1931) Plants, including fungi, poisonous or otherwise injurious to man in Australia. *Medical Journal of Australia*, **ii**, 775.

Cobb, H.M. (1930) Sensitivity due to Christmas trees. *Journal of Allergy*, **1**, 442.

Cocker, W., Dahl, T., Dempsey, C. & McMurry, T.B.H. (1962) Extractives from *Andira inermis*. *Journal of the Chemical Society*, 4906.

Codd, L.E.W. (1951) *Trees and shrubs of the Kruger National Park*. Dept. of Agriculture, Pretoria.

Cohen, H.I., Merigan, T.C., Kosek, J.C. & Eldridge, F. (1967) Sequoiosis: a granulomatous pneumonitis associated with redwood sawdust inhalation. *American Journal of Medicine*, **43**, 785.

Cooke, R.G. & Dowd, H. (1952) Naphthoquinones from *Diospyros hebecarpa*. *Australian Journal of Scientific Research*, **5A**, 760.

Cordero, A. & Lynch, H. (1951) Sensibilizacion profesional al aserrin de Lapacho. *Prensa Medica Argentina*, **38**, 838.

Corner, E.J.H. (1952) *Wayside Trees of Malaya*, 2nd edn. Govt. Printing Office, Singapore.

Cornevin, C. (1887) *Des plantes vénéneuses*. Firmin-Didot, Paris.

Crocker, H.R. (1903) *Diseases of the Skin*, 3rd edn, p. 418. Lewis, London.

Crow, K.D. (1970) Chloracne. *Transactions of the St John's Hospital Dermatological Society*, **56**, 79.

Czimatis & Hagemann (1910) Ueber Erkrankungen durch Holzstaub. *Hygienische Rundschau*, **20**, 761.

Dantin-Gallego, J. & Armayor, A.F. (1952) Some new toxic woods: some new manifestations of toxicity. *Industrial Medicine and Surgery*, **21**, 41.

Dassler, H.-G. & Urzynicok, H. (1958) Uber den Gerbstoff der Schwarzerle (*Alnus glutinosa* Gärtn.). *Holz als Roh- und Werkstoff*, **16**, 327.

Davidson, J.M. (1941) Toxic effects of Iroko, an African wood. *Lancet*, **i**, 38.

Davis, G. (1970) Imported hardwood statistical trends. *Timber Trades Journal*, 28.2.70, pp. 31–34.

Davy, J.B. (1929) African 'Sandaleen' wood. *Tropical Woods*, **17**, 15.

Dawson, C.R. (1954) The toxic principle of Poison Ivy and related plants. *Record of Chemical Progress*, **15**, 39.

Dempsey, C.B. & Donnelly, D.M.X. (1963) Extractives of *Dalbergia latifolia*. *Chemistry and Industry*, 23.3.63, p. 491.

Denis, P. (1937) Un nouvel alcaloïde des Rubiacées: la rubradinine. *Bulletin de l'Académie Royale Belgique* (classe Sciences) 5e série, **23**, 174.

Denis, P. (1938) La mitraspécine, nouvel alcaloïde du *Mitragyna speciosa* Korthals. *Bulletin de l'Académie Royale Belgique* (classe Sciences) 5e série, **24**, 653.

Dietrichs, H.H. (1958) Importance des constituants des bois pour leur utilisation. *Bois et Forêts des Tropiques*, **62**, 37.

Dietrichs, H.H. (1964) Desoxylapachol in *Tabebuia* sp. *Naturwissenschaft*, **51**, 408.

Dixon, W.E. (1911) The pharmacological action of South African Boxwood (*Gonioma kamassi*). *Proceedings of the Royal Society series B.*, **83**, 287.

Doerr, R. (1913) Allergie und Anaphylaxie. In: *Handbuch der pathogenen Mikroorganismen* (Ed. by W. Kolle and A. Wassermann), 2nd edn. vol. 2 (ii), p. 954. Fischer, Jena, Berlin.

Doig, A.T. (1949) Other lung diseases due to dust. *Postgraduate Medical Journal*, **25**, 639.

Donnelly, D.M.X., Nangle, B.J., Prendergast, J.P. & O'Sullivan, A.M. (1968) Isolation of R-5-o-methyllatifolin from *Dalbergia cochinchinensis* Pierre. *Phytochemistry*, **7**, 647.

Donnelly, B.J., Donnelly, D.M.X., O'Sullivan, A.M. & Prendergast, J.P. (1969) The isolation and structure of melanoxin, a new dihydrobenzfuran from *Dalbergia melanoxylon*. *Tetrahedron*, **25**, 4409.

Downing, J.G. & Gurney, S.W. (1940) Dermatitis from cashew-nut oil. *Journal of Industrial Hygiene and Toxicology*, **22**, 169.

Dubreuilh, W. (1931) L'eczéma des bûcherons. *Annales de Dermatologie et de Syphiligraphie*, **7**, s. 2, 199.

Eade. R.A., Hinterberger, H. & Simes, J.J.H. (1963) Afrormosin (castanin, 6,4'-dimethoxy-7-hydroxyisoflavone) from *Castanospermum australe* Cunn. et Fras. *Australian Journal of Chemistry*, **16**, 188.

Earle, K.V. (1938) Toxic effects of *Hippomane mancinella*. *Transactions of the Royal Society for Tropical Medicine and Hygiene*, **32**, 363.

Eaton, K.K. (1973) Respiratory allergy to exotic wood dust. *Clinical Allergy*, **3**, 307.

Emanuel, D.A., Wenzel, F.J. & Lawton, B.R. (1966) Pneumonitis due to *Cryptostroma corticale* (maple bark disease). *New England Journal of Medicine*, **274**, 1413.

English, P.B. & Grey, L.P. (1943) Sap dermatitis and conjunctivitis caused by the wild fig (*Ficus tumila*). *Medical Journal of Australia*, **i**, 578.

Enzell, C. & Erdtman, H. (1957) The occurrence of Manool in *Cupressus sempervirens* L. *Acta Chemica Scandinavica*, **11**, 902.

Escarteffigue, M. (1935) Dermo-épidermite eczématiforme . . . de palissandre. *Bulletin de la Société française de Dermatologie et de Syphiligraphie*, **42**, 670.

Evans, W. (1905) A note on teak dermatitis. *British Journal of Dermatology*, **17**, 447.

Evelyn, J. (1664) *Silva: or, a Discourse of Forest Trees*. 5th edn (1729). London.

Eyton, W.B., Ollis, W.D., Sutherland, I.O., Gottlieb, O.R., Magalhaes, M.T. & Jackman, L.M. (1965) The Neoflavanoid group of natural products I–III. *Tetrahedron*, **31**, 2683.

Farmer, R.H. (1972) *A Handbook of Hardwoods*, 2nd edn. HMSO, London.

Feinberg, S.M. and Schoenkerman, B.B. (1940) Karaya and related gums as causes of atopy. *Wisconsin Medical Journal*, **39**, 734–736 and 786.

Filho, R.B. & Gottlieb, O.R. (1968) Oxiaininas A: Flavonas hexaoxigenades de *Apuleia leiocarpa*. *Anais da Academia Brasileira de Ciencias*, **40**, 151.

Findlay, G.H. (1963) Dermatitis of 'poison ivy' type from an indigenous S. African plant—*Smodingium argutum*. *South African Medical Journal*, **37**, 883.

Findlay, G.H., Whiting, D.A., Eggers, S.H. & Ellis, R.P. (1974) Smodingium (African 'poison ivy') dermatitis. *British Journal of Dermatology*, **90**, 535.

Findley, L.J. (1972) An unusual case of rosewood dermatitis of the genus Dalbergia (East Indian rosewood). *British Journal of Industrial Medicine*, **29**, 343.

Flecker, H. (1945) Injuries produced by plants in tropical Queensland. *Medical Journal of Australia*, **i**, 636.

Fleming, I. (1964) *You only live twice*. Cape, London.

Fombeur, J.-P. (1972) A propos de cas récents de tumeurs ethmoido-maxillaires chez les travailleurs du bois. *Archives des Maladies professionnelles*, **33**, 453.

Forsyth, A.A. (1968) *British Poisonous Plants*, 2nd edn, Bulletin 161, Ministry of Agriculture, Fisheries and Food. HMSO, London.

Fournier, E. (1965) Toxicité des herbicides et des protecteurs des bois. *Revue du Praticien*, **15**, 3793.

Foussereau, J., Benezra, C. & Ourisson, G. (1967) Contact dermatitis from laurel. *Transactions of the St John's Hospital Dermatological Society*, **53**, 141.

Fox, E.C. (1941) Mesquite wood dermatitis. *Archives of Dermatology and Syphilology*, **44**, 1098.

FPRL (1963/4) Forest Products Research Laboratory, Princes Risborough, England. Unpublished circulars on irritant timbers.

Fregert, S. & Rorsman, H. (1963) Hypersensitivity to Balsam of Pine and Spruce. *Archives of Dermatology*, **87**, 693.

Frei (1932a) Dermatitis durch afrikanisches Teakholz. *Dermatologische Wochenschrift*, **94**, 27.

Frei (1932b) Pockholzekzem infolge Sensibilisierung nach Trauma. *Zentralblatt für Haut- und Geschlechtskrankheiten*, **41**, 29.

Freise, F.W. (1932) Gesundheitsschädigungen durch Arbeiten mit giftigen Hölzern. Beobachtungen aus brasilianischen Gewerbebetrieben. *Archiv für Gewerbepathologie und Gewerbehygiene*, **3**, 1.

Freise, F.W. (1936) Vergiftungen durch Brasilianische Werkhölzer I, II. *Sammlung der Vergiftungsfällen*, 7 C, 1 & 61.

Freise, F.W. (1937) Vergiftungen durch Brasilianische Werkhölzer III. *Sammlung der Vergiftungsfällen*, 8 C, 13.

Frèrejacque, M. (1951) La mansonine, hétéroside digitalique de *Mansonia altissima*. *Comptes rendus de l'Académie des Sciences*, **233**, 1220.

Frison, E. (1948) Tecomahoutsoorten en Lapachol. *Pharmaceutisch tijdschrift voor België*, **25**, 33.

Gade, K. (1921) Ueber pneumokoniosen mit Asthma bei Holzsägerei-arbeitern. *Münchener medizinische Wochenschrift*, **68**, 1144.

Galeffi, C., delle Monache, E.M., Casinovi, C.G. & Marini Bettolo, G.B. (1969) A new Quinone from the heartwood of *Mansonia altissima* Chev: Mansonone L. *Tetrahedron Letters*, **40**, 3583.

Galewsky (1905) Uber Eucalyptus-Dermatitiden. *Dermatologische Zeitschrift*, **12**, 36.

Gamble, J.S. (1902) *A Manuel of Indian Timbers*, 2nd edn. Reprinted by Bishen Singh Mahendra Pal Singh, Dehra Dun, 1972.

Gandevia, B. (1970) Ventilatory capacity during exposure to Western Red Cedar. *Archives of Environmental Health*, **20**, 59.

Gandevia, B. & Milne, J. (1970) Occupational asthma and rhinitis due to Western Red Cedar (*Thuja plicata*), with special reference to bronchial reactivity. *British Journal of Industrial Medicine*, **27**, 235.

Ganse, W. van (1968) Allergies respiratoires d'origine professionnelle provoquées par les bois exotiques. *Archives des Maladies professionnelles*, **29**, 205.

Ganzoni, M. (1929) Das Ekzem als Arbeitsschädigung und seine rechtliche Stellung in der Versicherung. *Schweizer medizinische Wochenschrift*, **59**, 1169.

Gayer, S. (1928) *Die Holzarten und ihre Verwendung in der Technik*, 3rd edn. Jänecke, Leipzig.

Gellért, E. & Witkop, B. (1952) Die Identität der Alkaloids Kamassin mit Quebrachamin. *Helvetica Chimica Acta*, **35**, 114.

Genders, R. (1972) *A History of Scent*. Hamilton, London.

Genner, V. & Bonnevie, P. (1938) Eczematous eruptions produced by leaves of trees and bushes. *Archives of Dermatology and Syphilology*, **37**, 583.

Gerard, J. (1597) *The Historie of Plants* (Ed. by T. Johnson, 1636). London.

Ghillini, C.A. (1954) Un lichene causa di Allergosi cutanea. *Rivista de Anatomia e Patologica Oncologia*, **8**, 755.

Gibson, R.J.H. (1906) The physiological properties of 'West African Boxwood'. *Biochemical Journal*, **1**, 39.

Gimlette, J.D. (1929) *Malay Poisons and Charm Cures*, 3rd edn. Churchill, London.

Golse, M.J. (1937) Les plantes toxiques des Colonies. *Bulletin des Travaux de la Société de Pharmacie de Bordeaux*, **75**, 102.

Gottlieb, O.R. & Mors, W.B. (1958) The chemistry of Rosewood. II: isolation and identification of cotoin and pinocembrin. *Journal of the American Chemical Society*, **80**, ii, 2263.

Gougerot & Blamoutier (1922a) Intolérance anaphylactique aux applications médicamenteuses externes . . . huile de cade. *Bulletin de la Société médicale des Hôpitaux de Paris*, **46**, 733.

Gougerot & Blamoutier (1922b) Dermite eczémateuse professionnelle du à la poudre de palissandre. *Bulletin de la Société médicale des Hôpitaux de Paris*, **46**, 739.

Gougerot & Delay (1932) Eczéma par sensibilisation au bois de palissandre. *Bulletin de la Société française de Dermatologie*, **39**, 1339.

Govindachari, T.R., Pai, B.R., Subramaniam, P.S., Rao, U.R. & Muthukumaraswamy, N. (1967) Constituents of *Mesua ferrea* L. I: Mesuaxanthone A and B. *Tetrahedron*, **23**, 243.

Grabham, M. (1905) Satinwoods and Dermatitis. *British Medical Journal*, **i**, 822.

Grana, P.C. (1946) Conjunctivitis and dermatitis due to 'beach apple'. Report of 13 cases. *Archives of Ophthalmology*, **35**, 421.

Greshoff, M. (1909) Phytochemical investigations at Kew. *Kew Bulletin*, **10**, 397.

Griffioen, K. (1949) Bevat Djeungdjinghout een giftige en irriterende Stof? *Tectona*, **39**, 175.

Gronemeyer, W. & Fuchs, E. (1967) Krankheiten durch inhalative Allergen-Invasion. In: *Lehrbuch der klinischen Allergie* (Ed. by K.Hansen and M.Werner). Thieme, Stuttgart.

Grossmann, J. (1910) Gesundheitsschädliche Hölzer. *Bayrische Industrielle Gewerbeblatt*, **96**(6), 51.

Grossmann, J. (1920) Gesundheitsschädliche Holzarten. *Der Holzkäufer* (Leipzig), **17**, 529, 535, 540 and 545.

Guibourt, N.J.-B.G. (1849) *Histoire naturelle des Drogues simples*. Baillière, Paris.

Hamel, du, H.L. (1811–1816) *Traité des Arbres et Arbustes qui se cultivent en France en pleine Terre*. Michel, Paris. New edition.

Hancock, W.V. & Swan, E.P. (1965) The petroleum ether soluble extractives . . . of Douglas fir. *Phytochemistry*, **4**, 791.

Hanslian, L. & Kadlec, K. (1964a) Timber and timber dust. *Pracovní lékařství*, **16**, 276.

Hanslian, L. & Kadlec, K. (1964b) Allergy caused by Larch wood. *Pracovní lékařství*, **16**, 362.

Hanslian, L. & Kadlec, K. (1966) Dřevo z hlediska hygienichéko (VII) Biologicky silně agresívní dřeviny. *Drevo*, **21**, 157.

Hart, G. (1955) *Timbers of South East Asia*. Timber Development Association, London.

Hausen, B.M. (1970) *Untersuchungen über gesundheitsschädigende Hölzer*. Thesis, Hamburg. (See also Hausen, 1973.)

Hausen, B.M. (1972) Untersuchungen zur Uberempfindlichkeit gegen Sucupira- und Palisanderholz. *Berufsdermatosen*, **20**, 1.

Hausen, B.M. (1973) *Holzarten mit gesundheitsschädigenden Ihnaltsstoffen*. DRW-Verlags-GmbH, Stuttgart.

Hausen, B.M., Simatupang, M.H. & Weitbrecht, U. (1971) Allergie gegen Bethabara-Holz. *Berufsdermatosen*, **19**, 324.

Hay, J. (1907) Special report on the condition of the workers employed in the manufacture of shuttles from African Boxwood. *Report of the Chief Inspector of Factories and Workshops*, London, pp. 266–268.

Hearst, P.J., Shamma, M., Dudock, B.S. & Shine, R.J. (1968) Greenheart alkaloids III: Sepeerine (ocoteamine) and Demararine. *Journal of Organic Chemistry*, **33**, 1229.

Heilig, P.M. (1957) Ziekten veroorzaakt door bewerking van zekere houtsoorten. *Vakblatt Meubelindustrie*, **38**, 1440.

Hering, H. (1966) Uber Arbeitsdermatosen in der holzverarbeitenden Industrie. *Dermatologische Wochenschrift*, **152**, 807.

Herzheimer, K. (1912) Ueber die gewerblichen Erkrankungen der Haut. *Deutscher medizinische Wochenschrift*, **38**, 18.

Heyl, U. (1966) Kontaktekzem bei Uberempfindlichkeit gegen Sucupira- und Palisanderholz. *Berufsdermatosen*, **14**, 239.

Hinckley, F.L. (1960) *Directory of the historic cabinet woods*. Bonanza, N.Y.

Hjorth, N. (1961) Eczematous allergy to balsams. *Acta dermato-venereologica*, **41** (Suppl. 46), 1.

Hjorth, N. (1965) Contact sensitivity to plants and balsams. *European Congress of Allergy, Acta dermato-venereologica*, pp. 65–79.

Hjorth, N., Fregert, S. & Schildknecht, H. (1969) Cross-sensitization between synthetic primin and related quinones. *Acta dermato-venereologica*, **49**, 552.

Hoffmann, H. (1926) Uber Hautentzündungen nach Teakholzbearbeitung. *Zentralblatt für Gewerbehygiene*, NF **3**, 333.

Hoffmann, H. (1928) Uber hautreizende Nutzhölzer. *Dermatologische Zeitschrift*, **53**, 293.

Holčík, L. (1959) Dermatitidy z Rhus vernicifera. *Československá dermatologie*, **34**, 93.

Holtzapffel, C. & Royle (1843) Descriptive catalogue of the woods commonly employed in this country for the mechanical and ornamental arts. An extract from: *Turning and mechanical manipulation*. Holtzapffel, London.

Hon. L.Y. (1967) Skin irritation caused by Tembusu (*Fagraea fragrans*). *Malayan Forester*, **30**, 234.

Horand, R. (1907) Mains de crocodile: dermatose professionnelle produite par le bois de chataigner. *Gazette des Hôpitaux*, **80**, 255.

Horner, S. & Wigley, J.E.M. (1936) A case of dermatitis venenata due to Mansonia wood (*Sterculiacea altissima*). *British Journal of Dermatology*, **48**, 26.

Hornsey, J.F. (1914) Dermatitis venenata due to Rungus. *British Medical Journal*, **i**, 759.

Hounam, R.F. & Williams, J. (1974) Levels of airborne dust in furniture making factories in the High Wycombe area. *British Journal of Industrial Medicine*, **31**, 1.

Howard, A.L. (1934) *A manual of the timbers of the world*, 2nd edn. Macmillan, London.

Howell, J.B. & Blair, D.S. (1950) Eczema of the hands from wooden-handled objects. *Archives of Dermatology and Syphilology*, **62**, 400.

Hublet, P., Sporcq, J., Oleffe, J. & Heylen, C. (1972) Les accidents cutanés et muqueux provoqués par les bois tropicaux. *Archives Belges de Médecine sociale, hygiène, médecine du travail et Médecine légale*, pp. 314–337.

Hurst, E. (1942) *The poisonous plants of New South Wales*. N.S.W. poisonous plants committee, Sydney.

Hurtado, I.de (1965) Contact dermatitis caused by the 'Manzanillo' (*Rhus striata*) tree. *International Archives of Allergy*, **28**, 321.

Hurtado, I.de (1968) Studies on the biological activity of *Rhus striata* ('Manzanillo'). 2. Skin response to patch tests in humans. *International Archives of Allergy*, **33**, 209.

Irvine, F.R. (1961) *Woody plants of Ghana*. Oxford University Press, London.

Iseki, K. (1932) Fall von Dermatitis acuta durch 'Aangot'-Baum (*Semecarpus venosa* Volk.). *Acta Dermatologica (Kyoto)*, **19**, 31.

Ito, K. (1963) Allergic disorders of upper respiratory tract by sawdust of *Thuja standishii* (report 3). *Journal of Science and Labour (Tokyo)*, **39**, 501.

Ito, K. (1964) An antigenic substance, beta-thujaplicin, in the steam-distillate of the sawdust of *Thuja standishii* Carr. *Medical Journal of Shinshu University*, **9**, 165.

Iwakawa, K. (1911) Uber das entzündungserregende Pulver des japanischen Nutzholzes 'Tagayasan'. *Archiv für experimentelle Pathologie und Pharmacologie*, **65**, 315.

Janson, P. (1953) Citrusfrüchte und Hauterkrankungen. *Zeitschrift für Haut- und Geschlechtskrankheiten*, **14**, 144.

Jay, B.A. (1950) *Timbers of West Africa*, 3rd edn. Timber Development Association, London.

John (1913) Dermatitis nach Teakholzbearbeitung. *Aerztliche Sachverständigen-Zeitung*, **8**, 170.

Jones, H.E. (1904) Acute dermatitis produced by satinwood irritation. *British Medical Journal*, **i**, 1484.

Jong, J.C.de, Lenstra, J.B. & Vermeer, J.H. (1951) Eczema due to the wood of Peroba da campos; isolation of the allergen. *Acta dermato-venereologica*, **31**, 108.

Kappes, L.O. (1948) Balsam as a cause of contact dermatitis in a florist. *Annals of Allergy*, **6**, 21.

Katz, A.E. (1946) *Spice Mill*, **69**, 46, 50. Quoted in *Chemical Abstracts*, **40**, 7525.

Katzenellenbogen, I. (1955) Caterpillar dermatitis as an occupational disease. *Dermatologica*, **111**, 99.

Keil, H., Wasserman, D. & Dawson, C.R. (1946) Mango dermatitis and its relationship to poison ivy hypersensitivity. *Annals of Allergy*, **4**, 268.

Kern, A.B. (1960) Contact dermatitis from cinnamon. *Archives of Dermatology*, **81**, 599.

King, F.E. (1953) The chemistry of wood extractives. *Chemistry and Industry*, 1325.

King, F.E. & Acheson, R.M. (1950) Afzelin (Kaempferol-3-rhamnoside), a new glycoside isolated from Doussié. *Journal of the Chemical Society*, 168.

King, F.E., Baker, J.A. & King, T.J. (1955) A saponin constituent of Makoré (*Mimusops heckelii*). *Journal of the Chemical Society*, 1338.

King, F.E., Clark-Lewis, J.W. & Forbes, W.F. (1955) EpiAfzelechin, a new member of the catechin series. *Journal of the Chemical Society*, 2948.

King, F.E. & Grundon, M.F. (1949, 1950) The constitution of chlorophorin, a constituent of Iroko, the timber of *Chlorophora excelsa*. *Journal of the Chemical Society* (1949) 3348; (1950) 3547.

King, F.E., Grundon, M.F. & Neill, K.G. (1952) Constituents of the heartwood of *Ferreirea spectabilis*. *Journal of the Chemical Society*, 4580.

King, F.E., Housley, J.R. & King, T.J. (1954) Coumarin constituents of *Fagara macrophylla*, *Zanthoxylum flavum* and *Chloroxylon swietenia*. *Journal of the Chemical Society*, 1392.

King, F.E., King, T.J. & Manning, L.C. (1953) The constitution of Jacareubin, a pyranoxanthone from *Calophyllum brasiliense*. *Journal of the Chemical Society*, 3922.

King, F.E., King, T.J. & Ross, J.M. (1954) The constitution of Arjunolic acid, a triterpene from *Terminalia arjuna*. *Journal of the Chemical Society*, 3995.

King, F.E., King, T.J. & Ross, J.M. (1955) Isolation of a new triterpene (Terminolic acid) from *Terminalia ivorensis*. *Journal of the Chemical Society*, 1333.

King, F.E., King, T.J. & Stokes, P.J. (1954) The structure of further new flavones occurring in Ayan (*Distemonanthus benthamianus*); Distemonanthin. *Journal of the Chemical Society*, 4587, 4594.

King, F.E. & Morgan, J.W.W. (1960) The constitution of Katonic acid, a triterpene from *Sandoricum indicum*. *Journal of the Chemical Society*, 4738.

Kligman, A.M. (1957) Poison ivy (Rhus) dermatitis. *Archives of Dermatology*, **77**, 149.

Knoche, H., Ourisson, G., Perold, G.W., Foussereau, J. & Maleville, J. (1969) Allergenic component of a liverwort: a sesquiterpene lactone. *Science, N.Y.*, **166**, 239.

Koelsch (1931) Die Begutachtung der gewerblichen Dermatosen im Sinne der Verordnung vom 11 Februar 1929. *Zentralblatt für Haut- und Geschlechtskrankheiten*, **37**, 1.

Komatsu, F. (1963) Respiratory allergy in woodworkers. 14th International Congress of Occupational Health. *Excerpta Medica*, **62**, 1748.

Krais, P. (1910) *Gewerbliche Materialkunde*, Vol. 1: Die Hölzer. Krais, Stuttgart.

Krogh, H.K. (1962) Contact eczema caused by true teak (*Tectona grandis*). *British Journal of Industrial Medicine*, **19**, 42.

Krogh, H.K. (1964) Contact eczema caused by true teak. *British Journal of Industrial Medicine*, **21**, 65.

Kuske, H. (1939) Experimentelle Untersuchungen zur Photosensibilisierung der Haut durch pflanzliche Wirkstoffe. *Archiv für Dermatologie und Syphilis*, **178**, 112.

Lagrange, H. (1923) Conjonctivite d'origine anaphylactique. *Presse médicale*, **31**, 112.

Laidlaw, R.A. & Smith, G.A. (1959) Heartwood extractives of some timbers of the family Moraceae. *Chemistry and Industry*, 1604.

Lamb, F.B. (1966) *Mahogany of tropical America*. University of Michigan, Ann Arbor.

Lancet (1896) Letters from 'H.N.A.' (i, 1113), 'M.B.' (i, 1193) and 'M.R.C.S. &c.' (i, 1265).

Lande, I.S.de la (1948) The alkaloids of *Cryptocarya pleurosperma*. *Australian Journal of Experimental Biology*, **26**, 181.

Landor, J.V. (1943) Dermatitis venenata caused by smoke. *British Journal of Dermatology*, **55**, 17.

Laslett, T. (1894) *Timber and timber trees*, 2nd edn, p. 226. Macmillan, London.

Le Coulant, M.P. & Lopès, G. (1956a) A propos des dermites des bois. *Journal médicale de Bordeaux*, **133**, 245.

Le Coulant, M.P. & Lopès, G. (1956b) A propos des dermites aux sciures de bois. *Bulletin de la Société française de Dermatologie et de Syphiligraphie*, **63**, 80.

Le Coulant, M.P., Texier, Maleville, Géniaux, Tamisier & Bancons (1966) L'allergie au frullania: son rôle dans la 'dermite du bois de chene'. *Bulletin de la Société française de Dermatologie et de Syphiligraphie*, **73**, 440.

Legge, T.M. (1905/6/7) *Reports of the Chief Inspector of Factories and Workshops*: (1905) 380, (1906) 32, (1907) 248. London. See also Hay (1907).

Leider, M. & Schwartzfeld, H.K. (1950) Allergic eczematous contact type dermatitis caused by cocobolo wood (*Dalbergia*). *Archives of Dermatology and Syphilology*, **62**, 125.

Levin, S.J. (1941) Cocobolo wood dermatitis. *Journal of Allergy*, **12**, 498.

Lewin, L. (1928) *Gifte im Holzgewerbe.* Stilke, Berlin.

Lewin, L. (1962) *Gifte und Vergiftungen.* Haug, Ulm.

Lewith, R. (1929) Palisanderholzdermatitis. *Zentralblatt für Haut- und Geschlechtskrankheiten*, **30**, 8.

Ligon, R. (1673) *A True and Exact History of the Island of Barbados.* Parker, London.

Lima, A.O. (1953) Uber das antigene Verhalten der Olharze einiger Gattungen der Familie Anacardiaceae. *International Archives of Allergy and applied Immunity*, **4**, 169.

Lindley, J. (1838) *Flora medica.* Longman, London.

Lipparoni, E. (1951) Dermatiti da zecche e dermatiti da sostanze vegetali. *Archivio italiano di Scienze Mediche Tropicali*, **32**, 913.

Livingood, C.S., Rogers, A.M. & Fitz-Hugh, T. (1943) Dhobie-mark dermatitis. *Journal of the American Medical Association*, **123**, 23.

Longin, L.A. (1929) Une triade éruptive provoquée par les essences forestières. *Annales de Dermatologie et de Syphiligraphie*, **10**, 178.

Loro, H.-A.-M. (1923) L'urticaire de l'Iroko. *Archives de Médicine et de Pharmacie Navales*, **114**, 136.

Loudon, J.C. (1844) *Arboretum et Fruticetum Britannicum*, 2nd edn. Longman, London.

Lullo, O.di (1928) Le 'Paaj', dermite provoquée par le quebracho rouge. *Comptes rendus des Séances de la Société de Biologie*, **99**, 1000.

Lullo, O.di (1934) 'El páaj', une nouvelle dermatite vénéneuse. *Revue sud-américaine de Médecine et de Chirurgerie*, **5**, 513.

McCabe, P.H., McCrindle, R. & Murray, R.D.H. (1967) Constituents of sneezewood, *Ptaeroxylon obliquum* (Thunb.) Radlk. Part 1: chromones. *Journal of the Chemical Society*, 145.

Mackee (1913) Dermatitis venenata from coco-bolo wood. *Journal for Cutaneous and Genito-urinary Diseases*, **31**, 582.

MacKenna, R.M.B. & Horner, S. (1954) Occupational skin diseases. In: *Industrial Medicine and Hygiene* (Ed. by E.R.A.Merewether). Butterworth, London.

McKennis, H. Jr., Hearst, P.J., Drisko, R.W., Thorndyke Roe Jr. & Alumbaugh, R.L. (1956) Isolation of a tetramethoxylated alkaloid from Demerara greenheart. *Journal of the American Chemical Society*, **78**, 245.

McLean, S. & Murray, D.G. (1970) Isolation of indole (beta-carboline), pyridine and indole-pyridine alkaloids from *Nauclea diderrichii*. *Canadian Journal of Chemistry*, **48**, 867–868.

MacMillan, H.F. (1956) *Tropical planting and gardening with special reference to Ceylon*, 5th edn. Macmillan, London.

MacPherson, J. (1925a) The toxicology of Eucalyptus oil. *Medical Journal of Australia*, **ii**, 108.

MacPherson, J. (1925b) Dermatitis caused by Queensland Maple. *Medical Journal of Australia*, **ii**, 542.

Maibach, H.I. & Johnson, H.L. (1975) Contact urticaria syndrome. *Archives of Dermatology*, **111**, 726.

Maiden, J.H. (1909, 1916, 1920) On some plants which cause inflammation or irritation of the skin. *Agricultural Gazette of New South Wales*, **20**, 1073 (1909); **27**, 39 & 212 (1916); **31**, 386 (1920).

Maiden, J.H. (1919) Notes on *Acacia*, no. IV. *Journal of the Proceedings of the Royal Society of New South Wales*, **53**, 219–220 (whole paper 171–238 + plates).

Majima, R. (1922) Chemische Untersuchung der verschiedenen natürlichen Lackarten. *Berichte der deutschen chemischen Gesellschaft*, **55**, 191.

Malespine, E. (1935) L'eczéma du bois. *Annales d'hygiène publique, industrielle et sociale*, **13**, 313.

Marini-Bettòlo, G.B., Casinovi, C.G. & Galeffi, C. (1965) A new class of quinones: sesquiterpene quinones of *Mansonia altissima* Chev. *Tetrahedron Letters*, **52**, 4857.

Markin, L.E. (1930) Boxwood sensitiveness. *Journal of Allergy*, **1**, 346.

Mascré, M. & Paris, R. (1939) Sur l'écorce de dô et ses propriétés digitaliques. *Bulletin des Sciences Pharmacologiques*, **46**, 145.

Matthes, H. & Schreiber, E. (1914) Uber hautreizende Hölzer. *Berichte der deutschen pharmacologischen Gesellschaft*, **24**, 385.

May, S.B. (1960) Dermatitis due to *Grevillea robusta* (Australian silk oak). *Archives of Dermatology*, **82**, 1006.

Meister, H. (1934) Ueber eine neue Aetiologie für Gesichtsekzeme. *Schweizerische medizinische Wochenschrift*, **ii**, 993.

Meneghini, C.L. & Rubini, G. (1958) Le dermatiti professionali nell'industrie dal legno. *Minerva dermatologica*, **33**, 122.

Merck (1968) *Merck Index.* Merck, Rahway, N.J.

Merrill, E.D. (1944) Dermatitis caused by various representatives of the Anacardiaceae. *Journal of the American Medical Association*, **124**, 222.

Milham, S. & Hesser, J.E. (1967) Hodgkin's disease in woodworkers. *Lancet*, **ii**, 136.

Miranda Bastos, A.D. & Matos Filho, A.de (1962) A 'Jacaranda' timber causing dermatitis. *Proceedings of the 5th Forestry Congress*, **3**, 1414.

Mitchell, C. (1970) Occupational asthma due to Western or Canadian red cedar (*Thuja plicata*). *Medical Journal of Australia*, **57**(ii), 233.

Mitchell, J.C. (1965) Allergy to lichens. *Archives of Dermatology*, **92**, 142.

Mitchell, J.C. & Armitage, J.S. (1965) Dermatitis venenata from lichens. *Archives of Environmental Health*, **11**, 701.

Mitchell, J.C., Dupuis, G. & Geissman, T.A. (1972) Allergic contact dermatitis from sesquiterpenoids of plants. *British Journal of Dermatology*, **87**, 235.

Mitchell, J.C., Fritig, B., Singh, B. & Towers, G.H.N. (1970) Allergic contact dermatitis from *Frullania* and Compositae. *Journal of Investigative Dermatology*, **54**, 233.

Mitchell, J.C., Schofield, W.B., Singh, B. & Towers, G.H.N. (1969) Allergy to *Frullania*. *Archives of Dermatology*, **100**, 46.

Moll, F. (1950) Giftiges Holz und Gewerbekrankheiten. *Holz (München)*, **4**, 76.

Montagu, Lady M.W. (1717) *Complete Letters* (Ed. by R. Halsband). Oxford University Press (1965). Letter to Lady Rich of 17.6.1717.

Morgan, J.W.W., Orsler, R.J. & Wilkinson, D.J. (1968) Dermatitis due to the wood dusts of *Khaya anthotheca* and *Machaerium scleroxylon*. *British Journal of Industrial Medicine*, **25**, 119.

Morgan, J.W.W. & Thomson, J. (1967). Ayan dermatitis. *British Journal of Industrial Medicine*, **24**, 156.

Morgan, J.W.W. & Wilkinson, D.S. (1965a) Dermatitis produced by wood. *International Union of Forestry Research Organisations Proceedings of Meetings* Section 41, **3**, 1.

Morgan, J.W.W. & Wilkinson, D.S. (1965b) Sensitization to *Khaya anthotheca*. *Nature*, **207**, 1101.

Mors, W.B., Gottlieb, O.R. & Djerassi, C. (1957) The chemistry of Rosewood. Isolation and structure of anibine and 4-methoxyparacotoin. *Journal of the American Chemical Society*, **79**, 4507.

Muenscher, W.C. (1951) *Poisonous plants of the U.S.A.*, 2nd edn (reprinted 1964). Macmillan, N.Y.

Murray, R.D.H. & Ballantyne, M.M. (1969) Nieshoutol, a sternutatory hydroxycoumarin from sneezewood. *Tetrahedron Letters*, **46**, 4031.

Nestler, A. (1911) Die hautreizende Wirkung des Amberholzes (*Liquidambar styraciflua* L.). *Berichte der deutschen botanischen Gesellschaft*, **29**, 672.

Nestler, A. (1912) Die hautreizende Wirkung des Cocoboloholzes. *Berichte der deutschen botanischen Gesellschaft*, **30**, 120.

Nestler, A. (1913) Die hautreizende Wirkung des roten Hartriegels und der Kornelkirsche. *Die Umschau*, **17**, 860.

Nestler, A. (1924) Hautreizende Hölzer. *Umschau*, **28**, 1007.

Nestler, A. (1926) Die hautreizende Wirkung des sogenannten 'Padouk-Holzes'. *Planta (Archiv für wissenschaftliche Botanica)*, **2**, 537.

Nestler, A. (1929) Hautreizende Pflanzen. *Umschau*, **33**, 611.

Neudörffer, R. (1902) Ein Fall von Vergiftung durch die Douglasfichte (*Tsuga douglasii*). *Centralblatt für innere Medizin*, **23**, 993.

Nicolas, J. & Pétouraud, C. (1935) Dermite eczématiforme du bras à la suite du port d'un bracelet d'ébène. *Bulletin de la Société française de Dermatologie et de Syphiligraphie*, **42**, 27.

Noll, R. (1931) Citronenholzmehl-Uberempfindlichkeit. *Zentralblatt fur Haut- und Geschlechtskrankheiten*, **38**, 437.

Nomenclature générale (1965) *Nomenclature générale des bois tropicaux*. Association technique internationale des bois tropicaux, Nogent-sur-Marne.

Nordin, J. (1947) *Yrkessjukdomar* (Industrial diseases), **2**, 605. Uppsala.

Nott, H. (1925) discussing Cleland (1925). *Medical Journal of Australia*, **ii**, 464.

Occolowitz, J.L. & Wright, A.S. (1962) 5-(10-pentadecenyl) resorcinol from *Grevillea puramidalis*. *Australian Journal of Chemistry*, **15**, 858.

Oehling, A. (1963) Berufsallergie im Holzgewerbe. *Allergie und Asthma*, **9**, 312.

Oesterle, O.A. (1913) Uber das 'Tekomin'. *Archiv der Pharmazie*, **251**, 301.

Oesterle, O.A. (1916) Uber einen Begleiter des Lapachols im Greenheartholz. *Archiv der Pharmazie*, **254**, 346.

Oliver, Thomas (1902) *Dangerous Trades*, p. 790. Murray, London.

Oliver, Thomas (1908) *Diseases of Occupation*, pp. 258–260. Methuen, London.

Opdyke, D.L.J. (1974) Monographs on Fragrance Raw Materials. *Food and Cosmetics Toxicology*, **12**, 807.

Oppenheim (1909) (Satinholzdermatitis) *Archiv für Dermatologie und Syphilis*, **96**, 97.

Oppenheim, M. (1913) Buchsbaumdermatitis bei einen Gärtner. *Osterreichisches Sanitätswesen*, **25**, 1291. Quoted by Hausen (1970).

Ordman, D. (1949a) Bronchial asthma caused by the inhalation of wood dust. *Annals of Allergy*, **7**, 492.

Ordman, D. (1949b) Wood dust as an inhalant allergen. Bronchial asthma caused by kejaat wood (*Pterocarpus angolensis*). *South African Medical Journal*, **23**, 973.

Orsler, R.J. (1969) The effects of irritant timbers. *Timberlab papers* no. 11. Forest Products Research Laboratory Princes Risborough.

Ortega, J.G. (1926) Amapa prieta. *Mexico Forestal*, **4**, 3 & 31. Quoted by Record in *Tropical Woods*, **7**, 37 (1926).

Pardo-Costello, V. (1923) Dermatitis venenata; a study of the tropical plants producing dermatitis. *Archives of Dermatology and Syphilology*, **7**, 81.

Pardy, A.A. (1954) Notes on indigenous trees and shrubs of Southern Rhodesia. *Rhodesia agricultural Journal*, **51**, 489.

Pautrier, L. (1903) Chrysarobin. *Revue pratique des Maladies cutanées, syphilitiques et vénériennes* Oct., Nov., Dec. 1903. Summary by A.Shillitoe in *British Journal of Dermatology*, **16**, 399 (1904).

Peckolt, T. (1873) Uber *Ipé tabaco* und seinen Gehalt an Chrysophansäure. *Zeitschrift des Allgemeinen Osterreichischen Apothekervereins*, **11**, 549.

Peckolt, T. (1909, 1913) Heil- und Nutzpflanzen Brasiliens. *Berichte der deutschen pharmazeutischen Gesellschaft*, **19**, 529; **22**, 24.

Pelikan, M.E. (1866) Nouvelles recherches sur le poison du *Nerium oleander*. *Comptes rendus de l'Académie des Sciences*, **62**, 237.

Pereira, M.O. de Silva, Gottlieb, O.R. & Magalhaes, M.T. (1966) A química de Gutíferas brasileiras. IX: Constituintes xantônicos do *Calophyllum brasiliense*. *Anais da Academia brasileira de Ciencias*, **38**, 425.

Peri, A. (1910) La lavorazione della corda di trucioli di pino nelle fonderie di tubi di ghisa. Le lesioni cutanee delle mani delle cordaie. *Il Ramazzini* (Firenze), **4**, 383. Quoted by Hausen (1970).

Perrins, J.D. (1852) Ueber das Vorkommen von Berberin in dem Columboholz von Ceylon (*Menispermum fenestratum* der Botaniker). *Annalen der Chemie und Pharmazie*, **83**, 276.

Pflanz, V. (1908) Interessante Fälle von 'Dermatitis venenata'. *Medizinische Klinik*, **4**, 832.

Philippe, J., Guibert, L. & Combes, R. (1965) Considérations sur les allergies sylvaines. *Revue française d'Allergie*, **5**, 95.

Phillips, E.W.J. (1966) Dermatitis caused by a rosewood substitute. *Wood*, **31**, 44.

Pillay, P.P. & Siddiqui, S. (1931) Chemical examination of the marking nut (*Semecarpus anacardium* L.). *Journal of the Indian Chemical Society*, **8**, 517.

Pillon & Martinet (1935) Sur huit cas de dermite par bois exotique. *Bulletin de la Société française de Dermatologie et de Syphiligraphie*, **42**, 380.

Piorkowski, F.O. (1944) Woodworker's dermatitis in East Africa. *East African Medical Journal*, **21**, 60.

Pirilä, V., Siltanen, E. & Pirilä, L. (1964) On the chemical nature of the eczematogenic agent in oil of turpentine. *Dermatologica*, **128**, 16.

Pliny (the elder). *Natural History* (Ed. by J.Bostock and H.T.Riley, 1855). Bohn, London, Vol. 3, p. 360. (Book XVI, chap. 20.)

Polonsky, J. & Lederer, E. (1959) Note sur l'isolement de la diméthoxy-2,6-benzoquinone des écorces et du bois de quelques Simarubacées et Méliacées. *Bulletin de la Société Chimique de France*, 1157.

Ponsinet, G. & Ourisson, G. (1965, 1968) Etudes chimio-taxonomiques dans la famille des Euphorbiacées. *Phytochemistry*, **4**, 813; **7**, 89.

Préfontaine, de (1763) *Maison rustique, à l'usage de Cayenne*, pp. 139, 152, 208–209. Bauche, Paris.

Ragot, A. & Brun, J.R. (1948) Les dermatoses professionnelles du bois d'Iroko. *9th International Congress of Industrial Medicine*, pp. 354–8. Wright, Bristol.

Ramazzini, Bernardino (1700) *De morbis artificum diatriba* (Ed. by A.Pazzini, 1953). Rome, Columbus. Chap. 6 in supplement.

Rasch, H. (1934) Poisonous woods. In: *Occupation and Health*, Vol. 2, pp. 680–685. International Labour Office, Geneva.

Raymond, V. (1959) Dermatoses professionnelles dans le bâtiment. *Revue du Praticien*, **9**, 2683.

Raymond, W.D. (1936) The poisonous effects of some local species of *Euphorbia*. *East African Medical Journal*, **12**, 369.

Record, M. (1945) A collection of woody plants from Melanesia. *Tropical Woods*, **81** (*Semecarpus*, p. 11).

Record, S.J. (1925) Lapachol. *Tropical Woods*, **1**, 7.

Record, S.J. (1926) (*Machaerium*) *Tropical Woods*, **8**, 18.

Record, S.J. (1929) Walnut woods—true and false. *Tropical Woods*, **18**, 4.

Record, S.J. & Garratt, G.A. (1923) *Cocobolo*. Yale University School of Forestry Bulletin no. 8. New Haven.

Record, S.J. & Hess, R.W. (1943) *Timbers of the New World*. Yale, New Haven.

RECORD, S.J. & MELL, C.D. (1924) *Timbers of Tropical America.* Yale, New Haven, and Milford/Oxford University Press, London.

RENDLE, B.J. (1956) Variation in the quality of African mahogany. *Wood*, **21**, 349.

RICHARD (1843) *Journal de Chimie médicale, de Pharmacie et de Toxicologie (2nd series)*, **9**, 392.

RICHET, C. (1909, 1910) Etudes sur la crépitine (toxine de *Hura crepitans Annales de l'Institut Pasteur, Paris*, **23**, 745; **24**, 609.

RIDLEY, H.N. (1922) Plant dermatitis. *Journal of tropical Medicine and Hygiene*, **25**, 225.

RIDLEY, H.N. (1924) Poisoning by bamboo hairs. *Journal of tropical Medicine*, **27**, 278.

RIDLEY, D.D., RITCHIE, E. & TAYLOR, W.C. (1968) Some further constituents of *Grevillea robusta* A.Cunn. *Australian Journal of Chemistry*, **21**, 2979.

RITCHIE, E. (1964) Chemistry of *Flindersia* species. *Review of pure and applied Chemistry*, **14**, 47.

RITCHIE, E., TAYLOR, W.C. & VAUTIN, S.T.K. (1965) Chemical studies of the Proteaceae: *Grevillea robusta* A.Cunn. and *Orites excelsa* R.Br. *Australian Journal of Chemistry*, **18**, 2015.

ROCHAIX, A. & VIEUX, G. (1936) Sur une dermatose provoquée par le 'bois d'olon'. *Revue d'Hygiène*, **58**, 590.

ROCKSTRO, R.S. (1890) *The Flute*, 2nd edn, pp. 141–142. Rudall Carte, London (1928).

SACHS, O. (1926) Beitrag zur Frage der Uberempfindlichkeit der Haut. *Wiener medizinische Wochenschrift*, **76**, 933.

SALAMONE, L., BLASI, S.DI & CONIGLIO, L. (1969) Rilievi sulla patologia da legno di Mansonia. *Folia medica (Napoli)*, **52**, 427.

SANDERMANN, W. & BARGHOORN, A.-W. (1955) Uber die Inhaltssoffe von Makoré- und Peroba-Holz sowie ihre gesundheitsschädigende Wirkung. *Holzforschung*, **9**, 112.

SANDERMANN, W. & BARGHOORN, A.-W. (1956) Gesundheitsschädigende Hölzer. *Holz als Roh- und Werkstoff*, **14**, 37 & 87.

SANDERMANN, W. & DIETRICHS, H.H. (1959) Uber die Inhaltsstoffe von *Mansonia altissima* und ihre gesundheitsschädigende Wirkung. *Holz als Roh- und Werkstoff*, **17**, 88.

SANDERMANN, W. & LANGE, W. (1967) Chemische Untersuchung des Holzes von Angélique (Basralocus—*Dicorynia guianensis* Amsh.). *Holzforschung*, **21**, 154.

SANDERMANN, W. & SIMATUPANG, M.H. (1961) Zur Chemie der Teaks, eines ungewöhnlichen Holzes. *Chemikerzeitung*, **85**, 38.

SANDERMANN, W. & SIMATUPANG, M.H. (1962) Ein toxisches Chinon aus Teakholz. *Angewandte Chemie*, **74**, 782.

SANDERMANN, W. & SIMATUPANG, M.H. (1966) Zur Chemie und Biochemie des Teakholzes. *Holz als Roh- und Werkstoff*, **24**, 190 (Review).

SANDERMANN, W., SIMATUPANG, M.H. & WENDEBORN, W. (1968) Neue Chinone aus dem Kernholz von *Paratecoma peroba* Kuhlm. *Naturwissenschaft*, **55**, 38.

ŠANTAVÝ, F. & REICHSTEIN, T. (1948) Evonosid, ein krystallisiertes, herzwirksames Glykosid aus *Euonymus europaea* L. *Helvetica Chimica Acta*, **31**, 1655.

SARGENT, C.S. (1896) *The Sylva of North America*, Vol. 10, p. 120. Houghton, Mifflin, Boston & New York.

SATULSKY, E.M. & WIRTS, C.A. (1943) Dermatitis venenata caused by the Manzanillo tree. *Archives of Dermatology and Syphilology*, **47**, 797.

SCADDING, J.G. (1967) *Sarcoidosis.* Eyre & Spottiswoode, London.

SCHEPERS, G.W.H. (1964) In: *Sensitivity Chest Diseases* (Ed. by M.C.Harris and N.Shure). Davis, Philadelphia. (Suberosis.)

SCHEVEN, H., GRONEMEYER, W. & FUCHS, E. (1963) Zum Antigenkatalog gewerblicher Inhalationsallergien. *Allergie und Asthma*, **9**, 286.

SCHIFF, B.L. (1951) Contact dermatitis caused by bamboo. *Archives of Dermatology and Syphilology*, **64**, 66.

SCHILDKNECHT, H., BAYER, I. & SCHMIDT, H. (1967) Struktur des Primelgiftstoffes. *Zeitschrift für Naturforschung*, B **22**, 36; 287.

SCHLUETER, D.P., FINK, J.N. & HENSLEY, G.T. (1972) Wood-pulp workers' disease- a hypersensitivity pneumonitis caused by *Alternaria. Annals of Internal Medicine*, **77**, 907.

SCHMIEG, K. (1926) Notes on new cabinet woods from Brazil. *Tropical Woods*, **5**, 1.

SCHOENTAL, R. (1973) Carcinogenicity of wood shavings. *Laboratory Animals*, **7**, 47.

SCHONWALD, P. (1929) discussing BLACK (1929). *Journal of Allergy*, **1**, 87.

SCHREIBER, M.M., SHAPIRO, S.I. & BERRY, C.Z. (1971) Cactus granulomas of the skin. *Archives of Dermatology*, **104**, 374.

SCHRÖPL, E. (1935) Eczema from granadilla wood. *Urological and Cutaneous Reviews*, **39**, 571.

SCHULMANN, E. & DÉTOUILLON, P. (1932) L'eczéma du bois et la sensibilisation sylvestre. *Paris Médical*, **i**, 55.

SCHULZ, K.H. (1957) Allergische Kontaktdermatitis durch exotische Hölzer, insbesondere durch Kambala-Teakholz. *Berufsdermatosen*, **5**, 238.

SCHULZ, K.H. (1962) Untersuchungen über die sensibilisierende Wirkung von Inhaltsstoffen Hölzer. *Berufsdermatosen*, **10**, 17.

SCHULZ, K.H. (1965) Allergische Kontaktdermatitis durch tropische Hölzer. *Dermatologia Internationalis*, **4**, 121.

SCHULZ, K.H. (1967) Berufsdermatosen — ausgewählte Kapitel. *Zeitschrift für Haut- und Geschlechtskrankheiten*, **42**, 499.

SCHULTZ, K.H. & DIETRICHS, H.H. (1962) Chinone als sensibilisierende Bestandteile von Rio-Palisander (*Dalbergia nigra*) und Cocobolo (*D.retusa*) Holz. *Allergie und Asthma*, **8**, 125.

SCHUR, A. (1932) Dermatitis venenata; report of a case due to the Osage orange. *Archives of Dermatology and Syphilology*, **26**, 312.

SCHWARTZ, L. (1931) Dermatitis venenata due to contact with Brazilian walnut wood. *Public Health Reports (Washington)*, **46**, 1938.

SCHWARTZ, L., BIRMINGHAM, D.J., CAMPBELL, P.C. & MASON, H.S. (1945) Skin hazards in the manufacture and use of cashew nut shell liquid formaldehyde resins. *Industrial Medicine*, **14**, 500.

SCHWARTZ, L., TULIPAN, L. & BIRMINGHAM, D.J. (1957) *Occupational diseases of the skin*, 3rd edn. Kimpton, London.

SCHWARZKOPF, K. (1954) Uber Hautschäden durch Holzschutzmittel. *Zeitschrift für Haut- und Geschlechtskrankheiten*, **16**, 6.

SCHWEISHEIMER, W. (1952) Wood dust and respiratory diseases. *Wood*, **17**, 181.

SENEAR, F.E. (1933) Dermatitis due to woods. *Journal of the American Medical Association*, **101**, 1527.

SÉZARY, A. & DEVAL, L. (1932) Dermite artificielle causée par des gants noirs. *Bulletin de la Société française de Dermatologie et de Syphiligraphie*, **39**, 1370.

SHERATON, T. (1803) *The cabinet dictionary*. London.

SHEVLYAKOV, L.V. (1969) On dermatitis caused by tropic species of wood. *Vestnik Dermatologii i Venerologii*, **43**, 73.

SIEGEL, J.M. (1954) Dermatitis due to black walnut juice. *Archives of Dermatology and Syphilology*, **70**, 511.

SIEGHEIM (1909) Ueber Satinholzdermatitis. *Berlin klinische Wochenschrift*, **46**, 2020.

SIEMENS, H.W. (1929) Strichförmige akute Dermatitis. *Dermatologische Zeitschrift*, **55**, 257.

SIMATUPANG, M.H., DIETRICHS, H.H. & GOTTWALD, H. (1967) Uber die hautreizenden Stoffe in *Vatairea guianensis* Aubl. *Holzforschung*, **21**, 89.

SIMMONS, J.S. & BOLIN, Z.E. (1921) Dermatitis venenata produced by an irritant present in the stem sap of the mango (*Mangifera indica* L.). *American Journal of Tropical Medicine*, **1**, 351.

SIMONS, R.D.G. (1953) *Handbook of tropical dermatology and medical mycology*, Vol. 2, pp. 1444–1452. Elsevier, Amsterdam.

SMITH, E.P. (1920) Plant dermatitis. *Journal of Botany*, **58**, 130.

SNOW, J.S. & HARLEY, R.D. (1944) Dermatitis venenata and keratoconjunctivitis caused by the manzanillo tree. *Archives of Dermatology and Syphilology*, **49**, 236.

SOSMAN, A.J., SCHLUETER, D.P., FINK, J.N. & BARBORIAK, J.J. (1969) Hypersensitivity to wood dust. *New England Journal of Medicine*, **281**, 977.

SOUSA, J.R. DE, GOTTLIEB, O.R. & MAGALHAES, M.T. (1967) Neoflavanoides mos gêneros *Goniorrhachis* e *Peltogyne*. *Anais da Academia brasileira de Ciencias*, **39**, 227.

SOWERS, W.F., WEARY, P.E., COLLINS, O.D. & CAWLEY, E.P. (1965) Ginkgo-tree dermatitis. *Archives of Dermatology*, **91**, 452.

SPIERS, P.S. (1969) Hodgkin's disease in workers in the wood industry. *Public Health Reports, Washington*, **84**, 385.

SPILLMAN, M.L. (1921) Dermite artificielle due à l'action du chêne. *Bulletin da la Société française de Dermatologie et de Syphiligraphie*, **28**, 33.

SPITZER, E. (1927) Gewerbedermatitis nach Okoméholz. *Zeitschrift für Haut- und Geschlechtskrankheiten*, **22**, 615.

SPRAGUE, T.A. (1921) Plant dermatitis. *Journal of Botany*, **59**, 308.

TANDLEY, P.C. (1927) Poisonous trees of Central America. *Tropical Woods*, **9**, 3.

TARKE, J.C. (1967) Photoallergy to sandalwood oil. *Archives of Dermatology*, **96**, 62.

TEINBRINCK, W. (1950) Besondere Beobachtungen an allergischen Krankheiten. *Zeitschrift für die gesamte innere Medizin*, **5**, 311.

STEINER, S.D. & SCHWARTZ, L. (1944) Dermatitis from mahogany wood (*Swietenia macrophylla*). *Industria Medicine*, **13**, 234.

, E. (1904) *Wayside and woodland trees*. Warne, London & N.Y.

Stern, E. (1891) Ueber einige Hautkrankheiten der Musiker. *Münchener medizinische Wochenschrift*, **38**, 739.

Sternberg, M. (1908) Aus dem Gebiete der Berufskrankheiten. *Medizinische Klinik*, **14**, 479.

Stewart, C.D. (1940) Dermatitis due to Mesquite wood. *Archives of Dermatology and Syphilology*, **42**, 937.

Stier, R.F.E. (1929) discussing Black (1929). *Journal of Allergy*, **1**, 88.

Stone, H. (1921) *A textbook of wood.* Rider, London.

Stone, H. (1924) *The timbers of commerce.* Rider, London.

Subiza, E. (1958) Pseudo allergia por maderas tropicales. *Medicina y seguridad del trabajo*, **6**, 74.

Subiza Martín, E. (1971) Etudes expérimentales avec le bois d'Oukola (*Dumoria africana*). *Les Bronches (Paris)*, **21**, 517.

Suskind, R.R. (1967) Dermatitis in the forest product industries. *Archives of Environmental Health*, **15**, 322.

Symanski, H.J. (1957) Opticusatrophie nach Verarbeitung von exotischen Hölzern? *Medizinische Klinik*, **52**, 198.

Tan, K.S. & Mitchell, J.C. (1968) Patch and photopatch tests in contact dermatitis and photodermatitis. *Canadian Medical Association Journal*, **98**, 252.

Tenchio, F. (1948) Etiologie de l'eczéma des bûcherons. *Dermatologica*, **97**, 72.

Thienemann, K. (1941) Kambala-Teakholz-Dermatosen. *Archiv für Dermatologie und Syphilis*, **182**, 551.

Thompson, W.G. (1914) *The occupational diseases.* Appleton, N.Y.

Timber Development Association (1963/4) Personal communication.

Titmuss, F.H. (1965) *Commercial timbers of the world*, 3rd edn. Technical Press, London.

Tommasi, L. (1929) Dermite dei boscaiuoli de ipersensibilità. *Giornale italiano di Dermatologie*, **70**, 1223.

Tottie, M. (1938) Jacaranda als Ursache von Dermatitiden. *Acta dermato-venereologica*, **19**, 235.

Touraine & Bernou (1932) Recherches expérimentales sur la dermatite du bois de 'Peroba amarella'. *Bulletin de la Société française de Dermatologie et de Syphiligraphie*, **39**, 1396.

Touraine & Golé (1934) Les dermatites par bois toxiques. *Paris médical*, **91**, 62.

Touraine, Hesse, J. & Golé (1932) Vingt cas de dermatite par bois satiné ('Peroba grande amarella'). *Bulletin de la Société française de Dermatologie et de Syphiligraphie*, **39**, 1392.

Touraine, A., Ménétrel, B. & Jouenne, M. (1934) Dermatite eczémateuse du palissandre. *Bulletin de la Société française de Dermatologie et de Syphiligraphie*, **41**, 1969.

Touton, K. (1932) Hauterkrankungen durch phanerogamische Pflanzen und ihre Produkte. In: *Handbuch der Haut- und Geschlechtskrankheiten* (Ed by J.Jadassohn), Vol 4/1, pp. 678–697 (woods). Springer, Berlin.

Toyama, I. (1918) Rhus dermatitis. *Journal of cutaneous Diseases*, **36**, 157.

Trousseau, A. (1886) La conjonctivite chrysophanique. *Annales de Dermatologie et de Syphiligraphie* (2nd series), **7**, 275.

Turc, H., Brunel, R. & Tolot, F. (1950) Accidents allergiques dus au bois de Makoré. *Archives des Maladies Professionnelles*, **11**, 490.

Vallander, A. (1950) The effect of wood dust on the lungs. *Nordisk Medizin*, **44**, 1315.

Volk, R. (1911) Das Uberempfindlichkeitsproblem in der Dermatologie. *Archiv für Dermatologie und Syphilis*, **109**, 163.

Vonkennel (1929) Teakholzdermatitis. *Zentralblatt für Haut- und Geschlechtskrankheiten*, **30**, 691.

Vorreiter, L. (1949) *Holztechnologisches Handbuch.* Fromme, Wien (later edition 1958).

Wagenführ, R. (1961) Gibt es giftige Hölzer? *Möbel und Wohnraum*, **4**, 120.

Walker, I.C. (1921) Bronchial asthma. In: *The Oxford Medicine* (Ed. by H.A.Christian), Vol. 2, p. 231. Oxford (1921–1947, loose-leaf).

Watt, J.M. & Breyer-Brandwijk, M.G. (1962) *The medicinal and poisonous plants of southern and eastern Africa*, 2nd edn. Livingstone, London.

Webb, L.J. (1948a) Guide to the medicinal and poisonous plants of Queensland. *Commonwealth Scientific and Industrial Research Bulletin* no. 232, Melbourne.

Webb, L.J. (1948b) The blistering alkaloid in 'poison walnut' (*Cryptocarya pleurosperma* Wh. & Fr.). *CSIR Forest Products News Letter* no. 169.

Weber, L.F. (1930) A list of cutaneous irritants. *Archives of Dermatology and Syphilology*, **21**, 761.

Weber, L.F. (1953) Dermatitis venenata due to native woods. *Archives of Dermatology and Syphilology*, **67**, 388.

Webster (1911) *New International Dictionary.* Merriam, Springfield.

Webster, A.D. (1920) *London Trees.* Swarthmore, London.

Wechselmann (1909) Ueber Satinholzdermatitis, eine Anaphylaxie der Haut. *Deutsche medizinische Wochenschrift*, **35**, 1389.

Wehrli, W., Schindler, O. & Reichstein, T. (1962) Die Glykoside des Milchsaftes von *Antiaris toxicaria* Lesch. aus Malaya sowie von *A. africana* Engl. aus Kenya. *Helvetica Chimica Acta*, **45**, 1183.

Weitbrecht, U. (1967) Allergie gegen Bethabara-Holz. *Berufsdermatosen*, **15**, 183.

WENDORFF, G.VON (1964) Personal communications. (Department of Federal Forest Research, Ibadan, Nigeria.)

WHITE, J.C. (1887) *Dermatitis venenata.* Cupples and Hurd, Boston.

WHITE, J.C. (1889) Some unusual forms of dermatitis venenata. *Boston medico-surgical Journal,* **121,** 583.

WHITE, J.C. (1903) Dermatitis venenata—a supplemental list. *Journal of cutaneous Diseases including Syphilis,* **21,** 441.

WHITE, R.P. (1920) *Occupational affection of the skin,* 2nd edn. Lewis, London.

WIESNER, J. (1903) *Die Rohstoffe des Pflanzenreiches,* 2nd edn. Engelmann, Leipzig.

WILKINSON, D.S. (1970) Dermatitis from Khaya mahoganies in the furniture industry. *Atti del Symposium* sulla *prevenzione delle dermatosi professionali* held at Monte Porzio Catone, 25/26 May 1970.

WILKINSON, D.S. (1971/3) Personal communications.

WINKLER, A. (1956) Dermatitis durch Pappelharz. *Dermatologische Wochenschrift,* **133,** 81.

WODEHOUSE, R.P. (1971) *Hayfever Plants,* 2nd edn. Hafner, N.Y.

WOOD, R.F. (1957) Robinia. In: *Exotic Forest Trees in Great Britain,* p. 164. Forestry Commission Bulletin no. 30 HMSO, London.

WOODS, B. (1962) Irritant plants. *Transactions of the St John's Hospital Dermatological Society,* **48,** 75.

WOODS, B. (1964) Unpublished survey of sawmills and woodworking factories in Ibadan, Nigeria.

WOODS, R.P. (1951) *Timbers of South America,* 2nd edn. Timber Development Association Ltd., London.

WOOLSTON, A. & PHILLIPS, E.J. (1955) Utilization of some of the woods in Alto Paraguay. *Tropical Woods,* **102,** 23.

YOSHIDA, H. (1884) Lacquer (*urushi*). Description, cultivation, and treatment of the tree, the chemistry of its juice, and its industrial applications. In: *Forestry and Forest Products* (Ed. by Rattray and Mill). Douglas, Edinburgh.

YOUNG, T.F. (1902) See OLIVER (1908).

YULE, H. & BURNELL, A.C. (1903) *Hobson-Jobson,* a glossary of Anglo-Indian (New edn. by W.Crooke). Reissued 1968. Routledge, London.

ZAFIROPOULO, A., AUDIBERT, L. & CHARPIN, J. (1968) Les accidents provoqués par la manipulation des bois exotiques. *Folia allergologica,* **15,** 396.

ZAVARIN, E., SMITH, R.M. & ANDERSON, A.B. (1959) Paper chromatography of the tropolones of Cupressaceae II. *Journal of Organic Chemistry,* **24,** 1318.

ZSCHUNKE, E. (1963) Krankheiten durch den Umgang mit Holz. *Holztechnologie,* **4,** 51.

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Toxic Woods by BRIAN WOODS and C. D. CALNAN

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